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DISINFECTION KINETICS OF BIOLOGICALLY TREATED DRINKING WATER AND THE IMPACT OF RELEASED GAC FINES ON DISINFECTION PERFORMANCE

BY

DAVID J. PERNITSKY

©

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Masters Of Science

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Disinfection Kinetics of Biologically Treated Drinking Water and the Impact of Released GAC Fines on Disinfection Performance** submitted by **David J. Pernitsky** in partial fulfillmentof the requirements for the degree of **Masters of Science** in **Environmental Engineering**.

Dr. Gordon R. Finch

Dr. Peter M. Huck

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Date: July 12, 1993

ABSTRACT

A physical / chemical desorption technique was used to assess the impact of colonized granular activated carbon (GAC) fines released from the biologically active carbon (BAC) contactors on disinfection performance. The procedure was found to be effective for the recovery of attached heterotrophic plate count (HPC) bacteria at GAC fines concentrations as low as 10 μg/L in experiments using fines colonized in the laboratory. Application of the desorption procedure to disinfected pilot plant effluents did not result in increased HPC recoveries. It was therefore concluded that biological treatment with BAC did not result in increased numbers of viable bacteria being introduced into the product water after disinfection. The disinfection kinetics of biologically and non-biologically treated drinking water effluents were evaluated at a pilot plant employing BAC. The disinfection kinetics of the biologically and non-biologically treated waters were found to be statistically different for both free chlorine and preformed monochloramine. However, the actual level of inactivation was statistically similar for both the biologically and non-biologically treated streams. Overall, free chlorine was more biocidal than monochloramine. The kinetics of all streams for both disinfectants deviated from pseudo-first order, with decreasing rates of inactivation seen at extended contact times. This tailing off effect, and the increased resistance to free chlorine found in the biological streams was thought to be due to the presence of a highly resistant subgroup within the HPC population.

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1. INTRODUCTION

This research is part of a larger study sponsored by the American Water Works Association Research Foundation (AWWARF) entitled The Design of Biological Processes for Organics Control (Contract No. 712-91). The work involved in this thesis was directed towards addressing possible negative impacts of biological drinking water treatment on effluent quality; namely any negative impacts as a result of the biological treatment on the final disinfection process. Water samples used in this research were collected from a pilot scale biological drinking water treatment plant operated by the University of Alberta at the City of Edmonton Rossdale Water Treatment Plant.

1.1. OBJECTIVES

The specific objectives for this research study were:

1. Evaluate the impact on disinfection of colonized fines released from the granular activated carbon (GAC) contactors during normal operation; and

2. Compare the general disinfection kinetics of biologically versus non-biologically treated water using both free chlorine and monochloramine as disinfectants.

1.2. SCOPE

To address the above objectives, a series of bench scale and pilot scale experiments were performed. In order to evaluate the impact of colonized fines released from the GAC contactors on disinfection, an experimental method was developed for the enumeration of bacteria attached to fines in the product water. Effluent from a bench scale GAC

column, and carbon fines colonized in the presence of heterotrophic plate count (HPC) bacteria, were used to develop the experimental method before it was applied to effluents from the pilot plant. The intent of the bacterial desorption experiments was to determine if any attached bacteria in the treated effluents surviving disinfection could be recovered and quantified. The intent of these experiments was not to estimate the number of GAC particles released from the contactors, nor to estimate the number of viable bacteria attached to the fines.

The disinfection kinetics of the biologically and non-biologically treated water samples were compared on the basis of a simple kinetic model. This kinetic model was developed for this study. It was not the aim of these disinfection studies to fit data to established kinetic models, or to fully define the kinetic behavior of biological effluents under all disinfection conditions. The comparison of disinfection behavior under a standard set of conditions was studied to determine any overall differences in the response of biologically and non-biologically treated effluents.

Disinfection and bacterial desorption experiments were conducted on the pilot plant effluents from November 1992 to February 1993. This period corresponded to the initial operating period of the biological treatment process. Because of the winter raw water temperatures, and the late October start up of the ozone system, this research was not conducted under conditions of maximum biological treatment. It is representative of winter river conditions at northern latitudes, which historically include lower temperatures and low raw water turbidities. The pilot plant

effluents were evaluated under normal operating conditions and pre- and post-backwash situations were not examined.

2. LITERATURE REVIEW

2.1. BIOLOGICAL DRINKING WATER TREATMENT

Biological treatment utilizes microorganisms to metabolize and subsequently remove undesirable compounds from water. This process has been used in Europe for a number of years for controlling bacterial growth, limiting disinfection byproduct (DBP) concentrations, reducing disinfectant demand, and removing taste and odor causing compounds and other micropollutants (Rittmann and Huck 1989). North America has been slow to embrace biological treatment because of a historical tradition of using chlorine disinfection to inhibit all microbial growth in water treatment plants, as well as more liberal DBP limits than in Europe. There are many advantages to biological treatment. Firstly, precursors of disinfection byproducts can be removed prior to final disinfection, thus avoiding the formation of harmful byproducts. As well, biological treatment removes a large portion of the biodegradable organic matter (BOM), providing a water with little substrate for bacterial regrowth in the distribution system. Possible disadvantages to the process include the release of bacteria into the treated water, and the production of bacterial toxins.

Process Schemes

The essential components of a biofilm based biological drinking water treatment system are a biodegradable substrate (or a substrate made more biodegradable by pre-oxidation), a biomass to degrade the substrate, and a media for the biomass to attach to. Several different pre-oxidants and filtration media have been used to treat drinking water in attached

growth biological systems. Slow sand filters, non-chlorinated rapid sand, dual media filters, fluidized bed filters, granular activated carbon (GAC) contactors, and in situ ground passage filters have all been shown to support biological growth (Bablon et al. 1988; Bouwer and Crowe 1988; Janssens et al. 1984; Rittmann and Huck 1989; Servais et al. 1991; Sontheimer 1978). Two of the more common process configurations for attached growth biological treatment are Biological Activated Carbon (BAC) and Biologically Active Filtration (BAF).

BAC

The Biological Activated Carbon (BAC) process refers to the combination of Pre-oxidation with ozone, and GAC filtration. Granular activated carbon filters have been used in the drinking water industry for the removal of taste and odor causing compounds, synthetic organic compounds, color forming organics, and disinfection byproducts and their precursors. Although it has been found that microbial activity develops naturally on GAC filters (AWWA Research and Technical Practice Committee on Organic Contaminants 1981; Bouwer and Crowe 1988), metabolism of biodegradable organics by these microbes was not originally a design objective.

Both deep bed GAC contactors and mixed media GAC/sand filters have been successfully tested, and in one specific study, their performance was found to be approximately the same (LeChevallier et al. 1992). No significant differences in effluent assimilable organic carbon (AOC) concentrations, bacterial counts, turbidity or headloss were found.

Because a mixed media contactor may be more easily retrofitted to current treatment plants, they may be more practical than deep bed contactors.

BAF

When ozonation is practiced upstream of non-GAC filters, increased biological activity within the filter occurs similar to that seen in BAC installations. However, systems employing filters containing GAC media tend to exhibit more biological treatment capability than filters with non-GAC media (AWWA Research and Technical Practice Committee on Organic Contaminants 1981; Bablon et al. 1988; Bouwer and Crowe 1988; Huck et al. 1991; LeChevallier et al. 1992). Reported increases in biodegradation rates on GAC relative to nonadsorbing media may be due to utilization of adsorbed substrate, higher surface area, or a more favorable acclimation environment (Bouwer and Crowe 1988).

A significant amount of treatment does, nevertheless, occur in BAF systems (LeChevallier et al. 1992). Results from a previous study at the Edmonton pilot plant, employing both BAF and BAC, reported that the effluent from dual media filters located upstream from BAC contactors were always found to have lower AOC P-17 concentrations than the raw water (Huck et al. 1991). A combined dual media / BAC process train may be useful in waters with high organic loading.

Pre-oxidation

Most biological process schemes include a pre-oxidation step which can enhance the assimilation of organic carbon by the biofilm in the filters, and therefore increase the biomass and BOM removal rate. Increases in

the biodegradable fraction of the organic material have been consistently reported immediately after pre-oxidation (Huck and Anderson 1992; Huck et al. 1991; Janssens et al. 1984; LeChevallier et al. 1992; Prevost et al. 1989). Oxidation of organic compounds in water usually produces oxygenated organic byproducts that are more biodegradable than the original compounds. The production of low molecular weight compounds from larger molecules which are resistant to biodegradation, e.g. humic and fulvic acids, is the main reason for this increased biodegradability (Janssens et al. 1984; van der Kooij et al. 1989). In general, without the pre-oxidation step, many of the benefits of biological treatment would not be realized, since the amount of naturally occurring biodegradable material in drinking water sources is often low. It is the two step process of oxidation / biodegradation that allows this process to remove such a wide variety of compounds, including those that are not naturally biodegradable.

There has been some debate over the best location in the treatment train for pre-oxidation. In France, ozone is applied after filtration and before GAC, since the reduction in turbidity by the filters is thought to aid the oxidation process. However, this results in biological activity in the GAC column only. Ozone can also be applied before filtration, with biological treatment occurring in both the filter and the GAC contactor. Because AOC removal in dual media filters has been demonstrated (Huck et al. 1991; LeChevallier et al. 1992), this arrangement should result in higher overall removals. Two stage biological treatment also lends itself well to plants with existing filter systems.

Although ozone is the oxidant most studied in conjunction with biological drinking water treatment, other oxidants have been investigated. Biological activity has been reported in the presence of both a free chlorine and a combined chlorine residual in the filters (LeChevallier et al. 1992). A comparison between pre-ozonation, prechlorination, and pre-chloramination (chlorine addition followed by ammonia addition), in this same study, found that pre-ozonation resulted in the highest effluent AOC levels, followed by pre-chloramination, and preoxidation with free chlorine.

Effluent AOC concentrations are not the only criteria for selection of a pre-oxidant. DBP concentrations were found to be higher in effluents exposed to prechlorination as opposed to pre-ozonation for trihalomethanes (THM), haloacetonitriles, and haloketones (LeChevallier et al. 1992). In addition, secondary increases in AOC following post disinfection were reported to be much less with pre-ozonation than prechlorination. These results reflect the greater oxidation potential of ozone. Refractory compounds that would form DBPs with chlorination are oxidized to biodegradable compounds with ozone, and removed biologically before DBPs are formed in the final disinfection step.

Impact of Colonized GAC Fines on Disinfection Performance

One of the primary concerns with BAC treatment is the breakthrough of microbially colonized carbon fines into the distribution system. Bacteria can been found attached to a wide variety of particles, in all phases of treatment, including: sediment particles in the raw water, floc fragments, filter sand particles, GAC fines, and the corrosion products found in

distribution systems. The release of fine GAC particles in the contactor effluent is well documented, and is thought to result from abrasion of larger grains during the backwash routine, structural weakening of the grains due to exposure to oxidants, and stresses induced by hydraulic loads (Camper et al. 1987; Camper et al. 1986; DiGiano et al. 1990; Stewart et al. 1988). In treatment facilities utilizing BAC, the problem of released fines is compounded by the fact that any released carbon particles are expected to have extensive biofilms attached to their surfaces, and can therefore introduce viable bacteria into the distribution system. Even in installations where the GAC contactors are exposed to water containing a disinfectant residual, micro-organisms have been recovered from released GAC fines (Stewart et al. 1988). In order to ensure product water that meets drinking water regulations, any attached microorganisms must be inactivated during the final disinfection step. However, numerous researchers have reported that organisms which are attached to particles show an increased resistance to disinfection (Berman et al. 1988; Camper et al. 1986; Herson et al. 1987; LeChevallier et al. 1988; LeChevallier et al. 1984; Stewart et al. 1988).

Bacterial activity on GAC

The attachment of microorganisms to GAC particles and released fines has been shown in many studies through scanning electron microscopy (SEM) (LeChevallier et al. 1984; Stewart et al. 1988; Weber et al. 1978). Such analyses have revealed that the biofilm formed on the surface of the carbon is nonhomogeneous and comprised of a loosely knit matrix of bacteria and protozoa often enveloped in an extracellular slime matrix. The surfaces of GAC particles provide a favorable environment for

bacterial colonization for several reasons (AWWA Research and Technical Practice Committee on Organic Contaminants 1981; Camper et al. 1985b; LeChevallier et al. 1984; Rittmann and Huck 1989; Stewart et al. 1988; Weber et al. 1978). The adsorptive properties of carbon enrich nutrient and oxygen concentrations and can remove disinfectant compounds and other inhibitory substances by adsorption before they are able to damage the microorganisms. Microbial attachment is also enhanced in the presence of the variety of functional groups found on the carbon surface. As well, the porous surface of the carbon particles provides a protective environment from fluid shear forces, resulting in the highest concentrations of bacteria being found in these macropores and surface irregularities (LeChevallier et al. 1984).

The bacterial population found on the surface of GAC particles is diverse, and will vary at each location due to differences in source water and variations in treatment and operation. Some of the organisms isolated from GAC include: *Pseudomonas, Flavobacterium, Acinetobacter, Staphylococcus, Klebsiella, Escherichia coli,* and *Enterobacter* (Camper et al. 1986; Stewart et al. 1988). The isolation of human pathogens from carbon particles is less common, since it is believed that the extent of colonization by pathogens is limited by competition for nutrients and space from indigenous organisms within the contactors (Camper et al. 1985b). Typical biofilm densities on GAC particles are summarized in Table 1. As can be seen, densities may vary under different conditions.

Release of fines in effluent.

Particles of GAC with attached bacteria can penetrate post treatment processes and have been found in distribution systems (Bouwer and Crowe 1988; Camper et al. 1987; Camper et al. 1986). In order to determine the impact of these fines on the final water quality, estimates of both the release rate of these fines, and the populations of bacteria attached to them are needed.

In a detailed study of fines released from a pilot scale GAC contactor pre-oxidized with ClO_2, approximately 36 carbon particles (standard deviation 17; range 10 to 62) were released per litre of GAC column effluent (Stewart et al. 1988). The particles ranged in size from 2 to 40 μm in diameter, with a mean diameter of 5.4 μm (standard deviation 4.6). A similar study of a conventional, bench scale GAC/sand filter found 10 to 30 particles/L were released, corresponding to less than 10 μg/L of carbon (DiGiano et al. 1990). As many as 400 particles/L were measured in a pilot scale installation in the same study. However, the authors believed that these elevated numbers were, in part, due to carry over of powdered activated carbon (PAC) particles from an upstream process.

A comparison of three filter media found that similar quantities of fines were released by GAC and anthracite filters (Camper et al. 1987). There was no difference in the numbers of HPC associated with the GAC and anthracite fines, although more coliform organisms were found on the GAC particles. Sand filters released more fines than either of the other media, but each particle supported fewer attached organisms. Others

have also indicated that more fines are released by sand filters than GAC/sand filters (DiGiano et al. 1990).

A study involving the release of GAC fines from nine operating GAC contactors employing prechlorination, reported that greater than 40% of the samples contained fines colonized by HPC bacteria, and over 17% of the samples contained fines with attached coliform bacteria (Camper et al. 1986). These fines were sampled before the final disinfection step in each case. Stewart (1990) reported that the number of viable bacteria attached to released GAC fines ranged from 0 to 7 colony forming units (CFU) per particle, with a mean of 3 (standard deviation 3), as determined by a physical/chemical desorption technique (Camper et al. 1985a). Examination of other particles from the same pilot plant by SEM indicated that 8% had no colonization, 77% had 1 to 50 cells, 7% had 51 to 100 cells, and 8% had hundreds or thousands of cells (Stewart et al. 1988). Because of the asymmetrical distribution of colonized particles, and the inability of the SEM to determine viability, a mean level of viable HPC could not be determined by the microscopic examination. Based on the desorption results and the numbers of fines released from the contactors, Stewart estimated that between 0 and 434 viable attached bacteria (mean, 108; standard deviation, 54) were released per liter of final effluent, prior to final disinfection. Based on an enumeration of released fines at 10 µg/L, and an assumed biofilm density of 10^6 CFU/g carbon, others estimated that only 10 CFU/L of viable attached bacteria would be released in the product water (DiGiano et al. 1990). The results of several studies on the rate of release of fines and attached bacteria from GAC contactors are summarized in Table 2.

The rate of fines release is also affected by operational factors. Increased bed depth, higher applied water turbidity, and increased filtration rate can result in a higher number of released particles, an increased HPC bacterial load on the particles, and an elevated number of attached coliforms (Camper et al. 1987). Conflicting results have been reported for the effect of backwash on fines release. Decreases in effluent quality in granular media filters are common immediately after backwash (Amirtharajah and Wetstein 1980). Similarly, fines release rates from a bench scale GAC/sand filter were found to increase 100 times immediately after backwash (DiGiano et al. 1990). However, others have reported that populated GAC fines are released randomly throughout the entire filter cycle, with no peaks in breakthrough occurring immediately prior or after backwash (Camper et al. 1986). To control fines release, these researchers recommended the minimization of filter rate changes and influent turbidity, and higher efficiencies in backwashing. In addition, filters should be run to waste for a period after backwashing to prevent fines from entering the distribution system.

Disinfection of attached bacteria

Disinfection resistance of attached bacteria

Because the introduction of viable bacteria into the distribution system can potentially compromise public health, the disinfection resistance of bacteria attached to GAC particles is very important. Several investigations into the matter have been conducted. Most of these investigations have been carried out using intact pieces of filter media, either removed from contactor beds or artificially colonized in the

laboratory (LeChevallier et al. 1988; LeChevallier et al. 1984; Stewart et al. 1988). Fewer studies have been performed on actual fines recovered from contactor effluents (Camper et al. 1987; DiGiano et al. 1990).

In one study, carbon particles from an operational water treatment plant colonized with naturally occurring HPC were chlorinated with 2 mg/L free available chlorine (FAC) (LeChevallier et al. 1984). After 1 hour of contact (1.7 mg/L FAC residual remaining), no significant decrease in viable cells was observed. The viability of unattached cells, however, decreased by more than 5 log units within 5 minutes. In the same study, similar results were obtained for *E. coli*, *Salmonella typhimurium*, *Y. enterocolitica*, and *Shigella sonnei* grown on carbon particles for 48 hours in the laboratory. When exposed to 1.4 to 1.6 mg/L FAC for 1 hour, no significant decrease in the viability of these bacteria was observed. In a separate investigation, exposure of GAC granules to 5 mg/L FAC for 1 hour resulted in HPC inactivations of only 58%, although at this chlorine concentration, considerable deterioration of the particles occurred (LeChevallier et al. 1988). Stewart (1988) found that after disinfection with 1.5 mg/L FAC and 1.5 mg/L NH_2Cl at pH 7, HPC bacteria attached to intact GAC granules were reduced by less than 0.5 log after a contact time of 40 minutes.

The inability of free chlorine to disinfect attached bacteria has also been reported for GAC fines (Camper et al. 1987). Released GAC fines were collected from two operational water treatment plants by attaching a gauze filter mechanism to sample ports on the GAC contactors prior to final disinfection. The concentrated particles were then removed from the filters and exposed to 2 mg/L FAC for 30 minutes. Attached coliform

bacteria were recovered from 32% of the disinfected samples. The concentration of fines present in the suspension during disinfection was not specified. This last point is quite important, since there is some evidence to suggest that GAC fines may not be difficult to disinfect if they are present in the concentrations expected under normal operating conditions (DiGiano et al. 1990). Based on preliminary results, DiGiano et. al. reported that suspensions prepared from particles collected in the product water of a filter adsorber were efficiently disinfected at these low concentrations (in the order of 10 μg/L). Disinfection was inhibited at particle concentrations in the order of mg/L. DiGiano et. al. hypothesized that since the carbon fines actually released are smaller than the crushed GAC particles used in other experiments, they may not contain as many attached bacteria per unit weight, may have lower binding power for bacteria, and may offer greater opportunity for diffusion of chlorine to the sites of attachment. (Released fines created by the fracture of a colonized granule of filter media may contain fewer attached bacteria per unit weight than the intact granule, since unless given sufficient time to re-attach, bacteria would not be expected to be present on the newly cleaved surfaces.) Although these results have not been verified, there is other evidence to support the hypothesis of DiGiano et. al. (1990). SEM analysis revealed that protrusions on the surface of GAC particles taken from an operational GAC installation were relatively free of bacteria (LeChevallier et al. 1984). This was thought to be due to wearing of the edges from several years of service. If this is an indication that bacteria require a crevice or crack to be adequately sheltered, then the fines found in BAC effluents may be less suitable for protection than intact particles. As well, it has been shown that smaller particles may provide less protection to

attached microbes. Coliforms associated with sewage particles < 7 μm in diameter were inactivated more rapidly by FAC (0.5 mg/L, pH 7) and monochloramine (1 mg/L, pH 8) than were coliforms associated with particles > 7 μm (Berman et al. 1988). However, due to the differences in particles, these results are not necessarily applicable to GAC fines.

Protection of microbes from disinfectant contact

Increased resistance to disinfection for bacteria attached to GAC particles is thought to be caused primarily by the protection from disinfectant contact afforded to the microbes. Microorganisms attached to, or associated with a wide variety of particles have been shown to be more resistant to disinfection due to the protection provided by the particles (Berman et al. 1988; Herson et al. 1987; LeChevallier et al. 1988; Ridgeway and Olson 1982). As discussed earlier, carbon surfaces are colonized by bacteria growing in cracks and crevices, and coated by an extracellular slime layer. Bacteria sheltered in this manner may never come into contact with the disinfectant, or may be embedded sufficiently that any biocide coming in contact with the carbon surface is adsorbed before it can inactivate the microbe. The degree of physical sheltering afforded by the GAC against chlorine contact can be illustrated by the results of LeChevallier (1988), in which exposure of GAC granules to 5 mg/L FAC for 1 hour resulted in HPC inactivations of only 58%, although at this chlorine concentration, considerable deterioration of the particles occurred.

The extracellular slime layer associated with biofilms may also play a role in protecting attached bacteria. Disinfection resistance of bacteria

attached to distribution system particles has been shown to increase as the length of time the microorganisms have been attached to the particles is increased from 2 to 18 hours (Herson et al. 1987). In another study (LeChevallier et al. 1984), washed *E. coli* cells were attached to GAC for 20 minutes; insufficient time for the formation of an extracellular slime layer to develop. No significant decrease in viability of attached cells was observed after 1 hour of exposure to a 1.4 to 1.7 mg/l FAC residual. However, a progressive increase in injury to the cells was observed. No injury was reported to *E. coli* cells allowed to form a slime layer, indicating that the slime layer was at least partially responsible for some disinfection sheltering.

Type of disinfectant

There is evidence to suggest that monochloramine may be a more effective disinfectant for biofilm organisms than free chlorine. At equal concentrations in the range of 1 to 5 mg/L, it has been reported that free chlorine and monochloramine result in similar inactivations of HPC attached to GAC particles (LeChevallier et al. 1988; Stewart et al. 1988). Similar trends were observed for *K. pneumoniae* attached to glass microscope slides (LeChevallier et al. 1988). When compared on an activity basis, pre-formed monochloramine has been reported to inactivate attached bacteria more efficiently than FAC (LeChevallier et al. 1988). Although attached organisms were found to be 150 to 3000 times more resistant to FAC than unattached cells, resistance of attached bacteria to monochloramine was increased by only 2 to 100 times compared to unattached cells.

The mechanism of action of monochloramine may account for its effectiveness against biofilm organisms. As was mentioned previously, monochloramine reacts rather specifically with protein containing substances such as amino acids, and does not react with sugars and extracellular polysaccarides. Therefore monochloramine is able to better penetrate the slime layer and kill the bacterial cells. Because chlorine is a less specific and a more powerful oxidant, it is more likely to be consumed before it penetrates into the biofilm.

Not all studies agree that monochloramine is a superior biocide for attached organisms. A study on the disinfection of coliforms associated with sewage particles found that the time required for 99% inactivation of the particle-associated bacteria with combined available chlorine (CAC) at pH 7 or 8 was 20 to 50 fold greater than the time required for the same amount of inactivation with chlorine at pH 7 (Berman et al. 1988). However, these particles were of a different nature than GAC.

Desorption of attached bacteria

Enumeration of bacteria in water samples containing colonized particulates is not possible using standard membrane filter or plating techniques. One of the underlying assumptions of these methods is that each visible colony is the result of a single organism (APHA AWWA WPCF 1989; Fisher et al. 1922; Haas and Heller 1986). This is not true in the case of colonized GAC particles, in which one particle may contain several viable organisms, but only produce one visible colony. In order to accurately determine the number of viable bacteria existing on the surface of the carbon, a method for removing these organisms from the surface is

necessary. For use in conjunction with disinfection experiments, it is necessary for the bacterial cells to remain undamaged by the desorption technique, so those organisms remaining viable after disinfection can be determined.

Several desorption methods have been used to remove attached bacteria from a variety of particles. These methods can be classified as physical, chemical, or a combination of these. Physical methods include shaking, blending, homogenization, and sonication. Shaking, either by hand or mechanically, relies on interparticle friction to remove loosely attached bacteria from particle surfaces, and has been used to remove bacteria from filter media (Jayadev and Chaudhuri 1990). Because the energy input for this method is limited, it is not very effective (Camper et al. 1985a). Blending and homogenization remove attached organisms by the generation of intense hydraulic shear forces. Because of the high energy input possible with these methods, they have been shown to be superior to shaking for colonized GAC particles (Camper et al. 1985a). Sonication is though to remove bacteria by disruption of the cell-particle bond. The drawback to this method of removal is that the removed bacteria may also be disrupted, and therefore impossible to enumerate by culturing techniques (Camper et al. 1985a; Ellery and Schleyer 1984). The addition of chemicals to suspensions of colonized particles has also been used to remove binding materials, neutralize surface charges, protect the cells, and prevent the reattachment of cells after physical removal (Camper et al. 1985a; DiGiano et al. 1990; Ellery and Schleyer 1984; McDaniel and Capone 1985).

A study comparing homogenization, sonication, and chemical desorption for the enumeration of bacteria sorbed to sediments, found that the efficiency of each physical technique varied with the type of sediment tested (McDaniel and Capone 1985). Chemical desorption was found to be less effective than either homogenization or sonication. Bacterial viability was not tested, as an epifluorescent direct enumeration technique was used. Others have found sonication to be superior to homogenization for enumerating attached sedimentary bacteria by an acridine orange direct count method (Ellery and Schleyer 1984). In a comprehensive evaluation of desorption techniques to desorb bacteria from GAC particles, Camper et. al. (1985a) found that homogenization was superior to sonication, blending, and shaking when it was also necessary to determine bacterial viability. Sonication was found to kill *E. coli* cells at all power levels tested. Elevated temperatures during blending also resulted in decreases in the recovery of viable bacteria. Homogenization at 16000 rpm for 3 minutes was found to recover the largest numbers of attached bacteria.

The addition of chemical surfactants was found to greatly increase the efficiency of homogenization procedures (Camper et al. 1985a). When known numbers of *E. coli* were attached to PAC and homogenized, only 4% of the population was recovered. It was assumed that the desorbed organisms were reattaching to new reactive sites on the carbon surface before enumeration was performed. Several compounds thought to be able to block this reattachment were tested, including: peptone, beef extract, gelatin, and tryptone. It was found that recovery of attached organisms increased to 83 to 94% when particles were homogenized in a

solution containing 0.01% peptone. The addition of enzymes thought to react with extracellular polymers did not appreciably increase bacterial counts. Further testing of solutions containing various surfactants and enzymes resulted in the greatest increases in HPC recovery when GAC particles were homogenized at 16000 rpm for 3 minutes (4 °C) in a solution of Tris buffer (0.01M, pH 7.0), Zwittergent 3-12 (10^{-6}M), ethyleneglycol-bis-(β amino-ethyl ether)-N,N^1-tetra acetic acid (EGTA, 10^{-3}M), and peptone (0.01%). The application of this procedure resulted in HPC recoveries 2.56 logs higher than by hand shaking alone. This desorption technique will be referred to as the Camper technique for the remainder of the text. The Camper technique has been successfully applied to studies involving the analysis of both GAC media particles (LeChevallier et al. 1988; LeChevallier et al. 1984), and released GAC fines (Camper et al. 1987; Camper et al. 1986; Stewart et al. 1988). Based on these studies, and recommendations of the original author (Camper 1992, personal communication), the following factors were determined to be, potentially, the most important in desorbing bacteria from fines:

1. desorption solution chemical concentration;
2. homogenization energy input; and
3. solution pH.

2.2. DISINFECTION

The disinfection process in drinking water treatment is one of the most important processes from a public health perspective, because of its role in minimizing the microorganisms responsible for diseases such as

cholera, infectious hepatitis, typhoid fever, and gastroenteritis (Craun 1988). With our greater medical understanding of the causes of waterborne disease we have become aware of pathogens other than the traditional enteric bacteria, such as *Giardia lamblia* and *Cryptosporidium parvum*. Some of these pathogens, such as *Giardia*, have proven to be very resistant to conventional chlorine disinfection (Hoff 1986). While increasing disinfectant concentrations does inactivate these resistant pathogens, it may be creating additional health risks (Sobsey et al. 1993). All of the disinfectants currently being considered for use in drinking water (including chlorine, monochloramine, chlorine dioxide, and ozone) are known to form some type of oxidation byproducts (Rice and Gomez-Taylor 1987). As well, both the disinfectants and their associated byproducts are known to have adverse health effects (Bull and Kopfler 1991). The most well known of these byproducts are the trihalomethanes (THM) formed by the reaction of free chlorine with the substances in raw water, such as humic and fulvic acids (Reckhow and Singer 1990; Reckhow et al. 1990). Other non-THM byproducts include: trichloroacetic acid (TCAA), dichloroacetic acid (DCAA), the haloketones (eg., 1,1,1-trichloroacetone or TCAC), and the haloacetonitriles (eg., dichloroacetonitrile or DCAN) (Reckhow and Singer 1990; Reckhow et al. 1990). The reduction in chlorinated disinfection byproducts (DBPs) is one of the driving forces behind research into biological drinking water treatment. By removing the DBP precursors through oxidation and biodegradation, effluent levels of DBPs can be reduced, compared with conventional treatment methods.

Chlorine Chemistry

Free Available Chlorine

Free available chlorine (FAC) exists in aqueous solutions as hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The relative proportions of these species vary with the water pH. The pKa for the dissociation of hypochlorous acid has been estimated at 7.582 at 20 °C (Morris 1966), and hydrolysis is considered to be complete under the conditions expected for drinking water applications. At pH 6.0, approximately 98% of the free chlorine is in the form of HOCl, while at pH 10.0, 99% is OCl^-. Between pH 7.0 and 8.5, the proportions of HOCl and OCl^- change rapidly, with HOCl decreasing from approximately 83% at pH 7.0, to approximately 14% at pH 8.5 (Hoff 1986). Because HOCl is generally recognized as being 150 to 300 times as biocidal as OCl^- (White 1972), the pH at which disinfection is carried out becomes very important. In addition, the biocidal efficiency of FAC is affected by the water temperature. Generally, the inactivation rate for FAC increases 1.5 to 2.5 times when the water temperature increases by 10 °C (Fair et al. 1948). For these reasons, all disinfection results must indicate both the water pH and temperature at which the experiments took place.

Monochloramine

Aqueous chlorine reacts with ammonia to produce a series of chlorinated ammonia compounds referred to as chloramines or combined available chlorine (CAC), as follows:

$$HOCl + NH_3 \leftrightarrow NH_2Cl \text{ (monochloramine)} + H_2O$$

$HOCl + NH_2Cl \leftrightarrow NHCl_2$ (dichloramine) $+H_2O$

$HOCl + NHCl_2 \leftrightarrow NCl_3$ (trichloramine) $+H_2O$

Further chlorination will eventually oxidize the ammonia to nitrogen gas or a variety of nitrogen-containing chlorine-free products which result in the familiar chlorine breakpoint reaction (Snoeyink and Jenkins 1980).

The chloramine reactions are governed primarily by pH and chlorine to nitrogen weight ratios, i.e., chlorine concentration relative to ammonia-nitrogen concentration usually expressed as Cl_2:N. Monochloramine is formed within seconds between the pH range of 7 to 9, and most rapidly at a pH of 8.3 (White 1972). Nearly all of the free chlorine is converted to monochloramine when Cl_2:N is $\leq$ 5:1, although a very small proportion of free chlorine remains. Synthesis of dichloramine can occur when pH is less than 5.5, provided that Cl_2:N is $\geq$ 5:1 (Wolfe et al. 1984). The formation of trichloramine occurs only at very low pH values (<4.4) or at high ratios of Cl_2:N ($\geq$ 7.6:1). In potable water systems, di- and tri-chloramines are generally found only after breakpoint chlorination (White 1972). Their formation is undesirable, since di- and tri-chloramines are associated with taste and odor problems (White 1972).

Free chlorine also reacts with nitrogenous organic compounds to form a variety of organic chloramines. These N-chloro organic compounds are undesirable byproducts of disinfection because they exhibit little or no microbiocidal activity, and because conventional techniques for measuring chloramine residuals cannot distinguish between inorganic chloramines and many organic chloramines (Wolfe et al. 1984).

Monochloramine is considered to be a very weak disinfectant. In general. 25 to 100 fold higher contact times are required with monochloramine to achieve the same degree of inactivation as a similar dose of free chlorine (Hoff 1986). The bactericidal activity of chloramines, as well as their speciation, is influenced by pH, temperature and Cl_2:N. In general, inactivation rates increase as the pH decreases, the temperature increases, and the Cl_2:N ratio increases. A study using both stock and environmental strains of several bacteria including *Escherichia coli,* reported that the rate of inactivation increased from 1.5 to 2 times as the chlorine to nitrogen weight ratio was adjusted from 2:1 to 5:1, and 5 to 6 times as the pH was decreased from 8 to 6 (Ward et al. 1984). Similar results using MS2 coliphage, *Escherichia coli,* and *Klebsiella pnuemoniae* have also been reported (Berman et al. 1992). These investigators hypothesized that the greater inactivation rates seen at high Cl_2:N ratios were due to the higher concentrations of FAC.

The most important variable affecting the biocidal potency of CAC solutions is the method of application. Three application schemes are generally used: prechlorination, concurrent addition of chlorine and ammonia, and pre-ammoniation. The inactivations reported in field applications of monochloramine are often higher than those predicted in the laboratory using preformed chloramines. In practice, chloramines are usually not preformed, and varying degrees of inactivation may actually be caused by the transient presence of free chlorine prior to its reacting with ammonia (Berman et al. 1992; Hoff 1986; Wolfe et al. 1984). Separate additions of free chlorine and ammonia (concurrent and pre-ammoniation) into seeded water at or below pH 7.5 were reported to

result in inactivation comparable to that observed with free chlorine (99% inactivation in less than 20 seconds). At pH 8, inactivation by separate addition was considerably slower, and was comparable to that by prereacted chloramines (99% inactivation in 25 to 26 minutes) (Ward et al. 1984). In a separate pilot plant study it was found that monochloramine prepared by concurrent addition and prechlorination resulted in statistically similar inactivations of HPC as free chlorination (FAC residual was approximately one half that for CAC) (Means et al. 1986). Inactivations with pre-ammoniation were significantly lower.

Disinfection Kinetics

For many reasons, it is desirable to treat pathogen disinfection in the same manner as a chemical reaction. Inactivation rates and the associated kinetic expressions allow comparisons of the biocidal efficiency of different disinfectants, determinations of the required doses for different conditions, and establishment of regulatory requirements based on a standard level of performance.

Historical development

Chick (1908) likened the inactivation of micro-organisms to a chemical reaction with the disinfectant and the protoplasm of the bacteria as the two reagents. Chick studied disinfection reaction rates by exposing bacteria to a constant concentration of disinfectant and enumerating the survivors at successive time intervals. The reaction velocity at any moment was found to be proportional to the number of organisms surviving at that moment, leading to the expression:

$$\log N/N_o = -kt$$

This relationship is analogous to that of a first order chemical reaction in which one of the reactants (in this case the disinfectant) is present in excess. It is important to remember when applying this pseudo-first order relationship that it only applies when the disinfectant concentration can be considered as a constant throughout the experiment. Ideally, plots of log N/No against t for various contact times should result in a straight line.

In actual experiments, however, this is not always the case. In fact, even in Chick's original work, only anthrax spores disinfected with phenol exhibited linear kinetics. Several species of bacteria did not follow the predicted first order kinetics, but instead they showed reduced reaction velocities as the experiments progressed, a phenomenon referred to as tailing off. These divergences were explained as being due to the varying resistance of different aged individual bacteria in the cultures, where the younger individuals were possessing higher resistance.

It is Watson (1908) who proposed a logarithmic relationship of the form:

$$k = C^n t$$

where k is equal to a constant for a specific microorganism exposed under specific conditions, and n is a constant referred to as the "coefficient of dilution". The plots of C and t on a double log scale are straight lines of slope n. Watson (1908) stated that the constants in the above relationship

are only valid at a constant temperature and may vary for different bacteria.

The coefficient of dilution, n, does not represent the order of the reaction, as it would for a chemical reaction. The coefficient n does not represent the total number of molecules of disinfectant that combine with the bacterial cell, but it represents the average number of molecules which have combined when the bacteria has become incapable of further growth. The role of n in the comparison of disinfection efficiencies under different conditions will be discussed further in section 2.2.

The incorporation of Watson's coefficient of dilution into Chick's kinetic expression results in the "Chick-Watson Law" (Haas and Karra 1984b):

$$\log N/N_o = -kC^n t$$

Theoretical concepts

In reality, the linear kinetics predicted by the Chick-Watson (C-W) model are rarely observed. Microorganisms are more complex and heterogeneous than reagent grade chemicals, and many different transport mechanisms and chemical reactions must occur in order to inactivate one organism (Jacangelo et al. 1991; Venkobachar et al. 1975; Venkobachar et al. 1977). Experimental conditions such as order of addition of disinfectant and microorganisms, mixing efficiency, and disinfectant demand, also influence the shape of the inactivation curve. A sampling of the types of curves generated in practice is shown in Figure 1.

Exponential Chick-Watson kinetics are shown in Figure 1A. Figure 1B shows concave upward kinetics or an "initial shoulder", followed by exponential kinetics. Concave downward kinetics are demonstrated in Figure 1C, and multiple or "tailing off" kinetics are shown in Figure 1D.

Deviations from linearity in disinfection kinetics are commonly attributed to either vitalistic effects or mechanistic effects. The vitalistic theory postulates that the deviations from the exponential law result from the inherent biological properties of the microorganisms, while mechanistic theory attributes these deviations to the chemical and physical factors occurring during the reaction (Hiatt 1964).

Mode of action for disinfectants

In general, vegetative bacteria exhibit a low resistance to chemical disinfectants. This tendency can be related to the physiological properties of bacteria, and the manner in which disinfectant compounds react with the microorganism. In 1948, the mode of action of free chlorine disinfection was believed to be a chemical reaction of HOCl with triosephosphate dehydrogenase, an enzyme system in the bacterial cell responsible for the utilization of glucose (Fair et al. 1948). The reaction was assumed to take place with the -SH groups of the enzyme, which are oxidized by chlorine. It was also believed that the rate of diffusion of the disinfectant through the cell wall determined the rate of disinfection. It is now thought that bacterial respiration takes place on the surface of the cell, with simple sugars and allied compounds on the outside of the cell wall, and enzymes, coenzymes, and H-carriers on the inside (Chang 1971). With these highly active systems present very close to the cell wall

biocidal agents, either as molecules or cations can react with greater ease with the H-carriers and coenzymes causing disruption of the respiratory process.

Detailed investigations into the mode of action of free chlorine have determined that the first step in inactivation is the reaction of chlorine with the cell membrane. Treatment with chlorine induces the leakage of macromolecules from the cells of *Escherichia coli*, indicating an increase in permeability in the membrane (Venkobachar et al. 1977). The role of the dehydrogenase enzymes in bacterial inactivation has been further elucidated since the work of Fair et. al. (1948). Chlorination has been shown to reduce the total dehydrogenase activity in intact *E. coli* cells. This was shown to be correlated with the overall kill of bacteria (Venkobachar et al. 1975). In addition, the succinic dehydrogenase (SDH) activity of the crude extract of *E. coli* also decreases rapidly within the bactericidal concentration of chlorine. The inactivation of SDH was hypothesized to be due to the oxidation of attached sulfhydryl groups. This dehydrogenase, along with several other enzymes is involved in the production of energy through oxidation reactions.

The mode of action of monochloramine is different than that for free chlorine in many respects. In an investigation into the mechanism of inactivation by monochloramine using *Escherichia coli*, it was found that bacterial cells were most likely killed by the inhibition of several basic transport and respiration systems (Jacangelo et al. 1991), rather than by reaction with a single cellular system, or cell lysing. The glucose and methionine transport systems were found to be the most sensitive to monochloramine, followed by the lactose and glycerol systems.

However, the rate of bacterial inactivation was found to be greater than the rate of inhibition for these transport systems, leading to the conclusion that their inhibition is contributory rather than solely responsible for cell death. As was the case for free chlorine, the total dehydrogenase activity was very sensitive to monochloramine. Again however, this system had a greater sensitivity than did overall bacterial viability. The inhibition of glucose oxidation was thought to be the primary mechanism of bacterial inactivation for monochloramine, as its inhibition was closely correlated to overall cell death. It is interesting to note that the biological inhibition of several systems was slower than the overall inactivation rate. This fact, coupled with the presence of a shoulder on the survival curves, prompted Jacangelo et. al. (1991) to conclude that monochloramine inactivation of *E. coli* involves a multiple hit mechanism.

In contrast to the mode of action for free chlorine discussed above, Jacangelo et. al. (1991) found that bacterial cells exposed to NH_2Cl do not evince gross structural damage or cellular lysis. As well, no damage to the cell membrane or leakage of intracellular material was reported in this study. It would appear from these results that monochloramine is able to pass through the cell membrane to attack internal systems.

Initial shoulder kinetics

Initial shoulder curves are usually encountered with more resistant types of microorganisms or weak biocides (Hoff 1986). Resistant microbes may have cell membranes of low permeability, causing the inactivation rate to be slowed until the disinfectant fully diffuses to the target system, after which the reaction proceeds exponentially. Alternately, a weak biocide

with a very slow reaction rate may delay the onset of linear kinetics in the early stages of the reaction. Hiatt (1964) stated that an induction period at the beginning of a disinfection experiment indicates either a complex reaction mechanism (mechanistic effects), or an inactivation mechanism based upon cumulative damage (vitalistic effects). The mechanistic explanation assumes the formation of intermediate compounds necessary to inactivate the organism. If the formation of the intermediate is slow, it will control the rate of inactivation during the early stages of the process. If the initial shoulder is consistently present when the experimental conditions are varied, a multiple hit mechanism (vitalistic) is probable. The basic assumptions in the multiple hit theory are that either a single target must be hit n times before it is destroyed, or that each organism contains n identical sites, each needing to be destroyed to kill the organism, and each requiring one hit to be destroyed. It has been postulated that the inactivation of *E. coli* by monochloramine is via a multihit mechanism (Jacangelo et al. 1991).

Tailing off kinetics

Decreases in the reaction rate with time are commonly seen in both chlorine and monochloramine disinfection (Berman et al. 1992; Chick 1908; Hoff 1986; Hom 1972; Sobsey 1989; Ward et al. 1984; Wolfe et al. 1985). Cerf (1977) stated that tailing off could be considered a normal feature of disinfection kinetics, and could be explained mechanistically by considering the mode of inactivation or resistance. For instance, the logarithmic kinetics predicted from a first order inactivation mechanism would not result if inactivation was caused by several first order reactions, or a higher order reaction. Likewise, the resistance of the

microbe may be modified during the treatment via a 'mutation', or an acquired tolerance to the disinfectant. This theory was also considered by Hiatt (1964), who stated that tailing off may be explained mechanistically if the particle resistance to disinfection increases with time, such as by reactions within the cell membranes decreasing the permeability and therefore preventing the disinfectant from penetrating into the cell and reaching the target system. Other mechanistic explanations include local variations in the treatment or mixing conditions during the experiments, clumping of organisms, disinfection depletion, or errors associated with enumerating the low numbers of microbes present at high levels of inactivation (Hoff 1986).

Multiphasic survival curves have often been explained by the presence of subgroups of microorganisms having varying degrees of resistance to the disinfectant. The change in slope of these curves may be abrupt or gradual, depending upon the nature of the microorganisms. A mixture of bacteria consisting of only two species with different inherent resistances would produce a curve with a very sharp kink, while gradual transitions in slope are due to either more than two distinct subgroups, or a population with a continuously distributed resistance to inactivation (Cerf 1977; Hiatt 1964).

Concave downward kinetics

Concave downward kinetics are seen less often than initial shoulder and tailing off effects. The possible causes for this effect include inadequate mixing or the extreme sensitivity of a small portion of the microbial population (Hoff 1986).

Modern Kinetic Models

A simple pseudo first-order model, such as the Chick-Watson model, is not adequate for describing all systems. Several alternative relationships have been proposed, including the Hom model (Hom 1972), Monod model (Haas 1980), Selleck model (Selleck et al. 1978), and two models by Chang (1971).

Hom

In order to account for variations in the reaction rate with time, such as initial shoulders and tailing off effects, Hom (1972) proposed the addition of an exponential constant, m, to the time variable in the Chick-Watson relationship. The Hom model can be expressed as follows:

$$\log N/N_o = -kC^n t^m$$

For the case of m equal to one, the Hom model is identical to the Chick-Watson model. For m values greater than 1, the inactivation curve displays an initial shoulder, while for m less than 1, a tailing off effect is seen. The degree of curvature is governed by the value of m as well. Gradually changing slopes result from m values close to one, while very small or large values result in inactivation curves with pronounced kinks.

Monod

Haas (1980) derived a kinetic model for inactivation which closely resembles the Monod model for microbial growth. The model, which was developed for the inactivation of virus by HOCl, can be used to describe

the disinfection kinetics of inactivation curves displaying initial shoulders. It is assumed that inactivation occurs as a two step process. Disinfectant molecules (C) are bound at receptor sites on the microbe (S) in a reversible manner, with the amount of bound disinfectant determining the rate of inactivation. It is assumed that there is a uniform number of binding sites per organism, β, and that both viable and killed microorganisms bind disinfectant in a uniform and constant manner, as shown below:

$$C + S \xleftrightarrow{k_1} CS \xrightarrow{k_2} \text{inactive microbe}$$

It is also assumed that disinfectant concentration is constant, and that the actual inactivation step is first order in viable microorganism concentration (N). The relationship is of the following form:

$$\ln N/N_o = \frac{-k_2 C\beta}{C + K_D}\left(t + \frac{e^{-k_1 t(C+K_D)} - 1}{k_1(C + K_D)}\right)$$

This equation has two very important properties. First, the equation predicts an apparent lag which is dependent upon concentration. Second, it predicts that the rate of inactivation, defined as the slope of the linear portion of the curve, can be given as a Monod-type function:

$$r_d = k_2 C\beta / (C + K_D)$$

The model was found to be a good descriptor for the disinfection of poliovirus in the original work.

Selleck

Selleck derived an empirical model to account for both the initial shoulders and tailing off effects commonly seen in drinking water and waste water disinfection (Selleck et al. 1978). This model attempts to explain the presence of a shoulder in the kinetic curve by assuming that no disinfection will occur until the product of contact time and concentration exceeds a minimum value. After this initial lag, inactivation occurs according to a declining rate. the Selleck model can be expressed as:

$N/N_o = 1$, for $Ct < b$, and;

$$N/N_o = \left(\frac{Ct}{b}\right)^{-n} , \text{ for } Ct > b$$

where (N/N_O) is the ratio of the surviving microorganisms to the initial number present; C is the disinfectant concentration; t is the contact time; and b and n are empirical constants. In contrast to traditional models in which plots of log survival versus time are used, the Selleck model has survival plotted against the product of concentration and time, on a double log scale. This results in curves with an initial portion of zero slope, followed by a linear, sloping portion. This procedure linearizes the tailing effect.

Chang

Two models were proposed by Chang to explain the disinfection kinetics observed in the presence of clumped bacteria (Chang 1971). Chang stated

that when survival curves show shoulders, indicating the presence of high percentages of organisms in clumps, the observed points can be fitted to curves generated by the multihit equation:

$$P_t = 1-(1-e^{-kt})^n$$

in which P_t is the probability of survival at time t; k is the destruction rate constant (usually computed from the linear section of the survival curve); and n is the number of organisms per clump.

Chang (1971) also postulated that the presence of small percentages of organisms in clumps is indicated when survival curves show tailing off. The steeper the initial drop in survivors, the lower the percentage of organisms in clumps. By considering a separate term for each different visible death rate present on the curve, the following equation was determined for analyzing such data empirically:

$$\frac{N_t}{N_o} = P_1 e^{-k_1 t} + P_2 e^{-k_2 t} + \ldots P_n e^{-k_n t}$$

in which N_t is the number of survivors (singles and clumps) present after time t; N_O is the total number of organisms (singles and clumps); P is the portion of N_O existing as singles for each region of the curve; and k is the death rate constant.

Comparison of models

Although many kinetic models have been proposed, and many explanations have been put forth to describe the non-linearity seen in disinfection data, the validity and usefulness of the Chick-Watson model

has changed very little. In a comprehensive review of disinfection data for free chlorine and preformed chloramine under demand free conditions, Haas and Karra (1984b) concluded that the Chick-Watson model produces an adequate fit to most data, and that no substantial improvement in the fit to experimental data can be obtained by the use of alternate models. Only in very few cases was there a statistically significant improvement in the correlation coefficients computed for either the Hom model, or the Monod model as compared to the Chick-Watson equation. However, the report also found that the residuals from the Chick-Watson estimation equation were greater than the experimental measurement errors associated with the disinfection data sets. This means that although the C-W model empirically fits the data, there is a need for improved understanding of disinfection theory.

One of the reasons that the alternate models are unable to provide globally better descriptions of disinfection kinetics, is that each of the models described above explains non-linearity on the basis of a single theory. The models developed by Chang (1971) are based upon applying the multihit theory to clumped bacteria. Chang (1971) commented that it appears to be technically incorrect to use the multihit theory (which describes hits that are simultaneous and related), for the inactivation of clumped bacteria in which the survival of one organism is independent of others in the clump. His approach was justified on the basis that survival curves with shoulders may be regarded as having a pseudo-multihit nature. The biggest drawback to the Chang equations is that while they may numerically describe some disinfection data well, they assume that the presence of organisms in aggregates is the only cause for aberrations

in survival curves. Most important in this regard is the fact that there is no provision for non-linearity due to heterogeneity of the bacterial population.

The Monod model is based on the assumption of an intermediate compound being formed between the disinfectant and the microorganism. The presence of variability in the resistance of the microbial population is not considered. The other vitalistic and mechanistic factors discussed above are also not addressed. Although this model may provide a better fit to some data, it is not broad enough to be used as a replacement for the Chick-Watson law.

Because of its similarity to the Chick-Watson law, the Hom model would appear to be a good candidate as a general kinetic relationship. After all, when m is equal to one, the Hom model and the C-W model are identical. None the less, both are empirical in nature and their mathematical constants do not represent the biochemical relationships between disinfectant and microbe. The addition of the constant m does allow the Hom model to better describe data sets featuring shoulders and tailing off. The fact that the review by Haas and Karra (1984b) did not find statistical advantages in the Hom model, may be an artifact of the data examined. The data sets used in the review, in general, included data with inactivations up to 2 or 3 logs only. Haas & Karra (1984b) acknowledged that in a more extensive investigation, with data extending to greater fractional inactivations, a less satisfactory agreement to the kinetic models may occur, particularly with respect to the tailing off phenomenon.

Although the Selleck model has no rational mechanistic basis in describing chemical disinfection, it empirically approximates the behavior of real systems. Data collected in both demand free systems, and systems with disinfectant demand have been successfully modeled by this relationship (Haas and Karra 1984a; Montgomery 1985), therefore making it a useful design tool.

2.3. INDICATORS OF DISINFECTION PERFORMANCE

Indicator Organisms

In order to ensure adequate disinfection of drinking water, utilities must monitor the microbiological quality of the treated water to ensure that it is effectively free of pathogens, and does not pose a risk to public health. Because of the large number of pathogens known to occur in drinking water, and because some are difficult to detect on a routine basis, a system of indicators has been adopted, in which selected groups of bacteria are used to indicate the potential for pathogen contamination. The ideal pathogen indicator for the evaluation of the bacteriological quality of water would (Montgomery 1985):

1. Always be present when pathogens are present;

2. Always be absent when pathogens are absent;

3. Be nonpathogenic; and

4. Be more resistant to disinfection and environmental stress than the pathogens.

The coliform group of organisms have traditionally been used as indicators, and coliform limits are still the basis of most drinking water regulations (U.S. Environmental Protection Agency 1989). The total coliform (TC) group includes bacteria from many species, including soil and enteric bacteria. The fecal coliforms (FC), a subgroup of the total coliform group, are used to represent organisms of fecal origin. *Escherichia coli* is the predominant bacterium in the fecal coliform group. Because coliforms are present in large numbers in fecal material, and in general are not human pathogens, they are thought to be a useful indicator for the possible presence of fecal contamination.

Several other microorganisms are also used as indicators. Fecal Streptococci (FS) are often used in conjunction with coliforms, as the ratio of FC to FS can be used to differentiate between human and nonhuman sources of contamination (Montgomery 1985). Because of their increased resistance to disinfection (Hoff 1986), phages and HPC bacteria may also be useful as indicators.

Traditional microbial indicators of water quality, such as total and fecal coliforms, may be inadequate for predicting the presence of several important bacterial, viral, and protozoan pathogens, primarily because these pathogens are more sensitive to disinfection than the indicators (Means et al. 1986; Sobsey 1989). Furthermore, current microbial indicator systems have not been evaluated or proven effective as indicators of conditional (opportunistic) pathogens, microbial toxins, allergens, and other agents of nonenteric health risks (Sobsey et al. 1993).

The heterotrophic plate count (HPC) bacteria are often used as indicators of the general microbial activity in water. This group consists of aerobic, organic carbon utilizing bacteria, grown on a non-selective media. Table 3 contains a partial listing of the bacteria species present in drinking water distribution systems. Any or all of these organisms may be present in a particular HPC population. The presence of high numbers of HPC is thought to indicate the presence of opportunistic pathogens, such as *Psuedomonas* spp., *Moraxella* spp., and *Flavobacterium* spp. (Geldrich et al. 1972; Lamka et al. 1980). The HPC population is also used by regulatory and standards organizations to indicate periods when the disinfectant residual is inadequate or ineffective for the removal of microorganisms of public health concern (APHA AWWA WPCF 1989; U.S. Environmental Protection Agency 1989).

Although in general, HPC bacteria are more resistant to disinfection than the coliform group, they are not ideal organisms for indicating the relative efficiency of disinfection processes. Because of their inherent heterogeneity, and the fact that the disinfection response of a particular HPC population is dependent on its speciation and the relative numbers of disinfectant tolerant organisms, HPC bacteria cannot be used as a regulatory tool. Several studies have found no correlation between HPC bacteria concentrations and water quality parameters such as coliform occurrence, turbidity, and disinfectant residual (Goshko et al. 1983; Haas et al. 1983; Reilly and Kippin 1983). In addition, conditional pathogens may be present in the HPC bacterial population, and allowing any concentration of these microbes in treated water may jeopardize public health (Sobsey et al. 1993).

HPC are, however, very useful in disinfection kinetics studies as they reflect the response of the naturally occurring organisms in their native state. These bacteria have been used in several studies to evaluate the disinfection efficiency of both free and combined chlorine (DiGiano et al. 1990; LeChevallier et al. 1992; LeChevallier et al. 1988; LeChevallier et al. 1984; Means et al. 1986; Ridgeway and Olson 1982; Stewart et al. 1988; Wolfe et al. 1985). The results of these studies indicate that HPC are relatively resistant to disinfection, and frequently exhibit tailing off kinetics. As well, because HPC concentrations are usually much greater than coliform concentrations, the use of HPC in kinetic studies allows results to be obtained at higher inactivation levels, where the largest tailing off effects occur.

It must be remembered that no matter which indicator system is used, the presence or absence of that indicator does not give absolute assurance of the presence or absence of pathogenic organisms. For some pathogens such as protozoan cysts, no indicator organism that shows comparable disinfection resistance and is easily monitored has been universally accepted. Consequently, until the methodology for detecting pathogens such as *Giardia lamblia* and *Cryptosporidium parvum* become practical for daily monitoring, we must rely upon some other parameter for ensuring adequate disinfection such as CT values, and surrogate organisms such as HPC.

The CT Concept

The objective of drinking water disinfection is to consistently inactivate pathogens to provide a microbially "safe" water. As was explained in

section 2.1, disinfection kinetics do not always follow accepted theories and are often difficult to predict. It is therefore the responsibility of regulatory agencies to provide a rational framework for disinfection procedures, to ensure that adequate inactivation is being provided by all utilities regardless of site conditions. In Alberta, disinfection is required for all surface water and shallow ground water supplies (Alberta Environment 1988). Disinfection efficiency is controlled by stipulating that a contact time of 20 minutes and either a free chlorine residual of 0.5 mg/L or a combined chlorine residual of 1.8 mg/L, must be maintained. No additional requirements are stated for waters of different pH or temperature. As part of the Surface Water Treatment Rule (SWTR) introduced in the United States by the U.S. Environmental Protection Agency (EPA), standards for chemical disinfection utilize the CT concept described previously (U.S. Environmental Protection Agency 1989). If a system can continually meet the tabulated CT values (disinfectant concentration multiplied by contact time), the system is considered to be in compliance with the regulation. For free chlorine, the CT values change with water temperature and pH. This section will discuss the basis for the application of this rule, as well as its advantages and disadvantages.

The CT concept was developed from the work of Watson (1908). CT values are determined according to the following equation:

$CT =$ a constant

where C is the concentration of a specific disinfectant (mg/L); T is the contact time (minutes) required for inactivation of a specific portion of the

microorganisms. The "CT value" is a constant for a specific microorganism exposed under specific pH and temperature conditions. It is critical to note that this equation does not include the coefficient of dilution, n, used in Watson's relationship.

Because the coefficient of dilution is not included in the CT expression, CT values are, in effect, calculated assuming that n equals 1. If this was indeed the case, CT values could be confidently used to extrapolate inactivation rates obtained experimentally at one concentration and contact time, to others, allowing the determination of the required contact time at higher or lower disinfectant residuals. There are two problems with applying the results of laboratory disinfection experiments to disinfection requirements in the field in this manner. First, the assumption that n is equal to 1 may underestimate the disinfection requirements for certain organisms. Secondly, for systems exhibiting non-linear kinetics, the extrapolation of laboratory data collected at relatively low levels of inactivation to the higher levels required in the field may also result in inadequate disinfection.

For the most part, n values for free chlorine tend to fall within the range of 0.7 to 1.3 (Haas and Karra 1984b). However, several values reported by the same authors were outside this range. The n values reported for *Giardia muris* with free chlorine varied from 0.34 to 4.76, under identical pH and temperature conditions. Similar results have been seen in the field. A study of the disinfection of native HPC bacteria with both FAC and CAC, found that changes in inactivation were not related to changes in disinfectant concentration (Wolfe et al. 1985), implying a very low n value. Figure 2 illustrates the effect of uncertainty in the coefficient of

dilution. If a CT value of 100 mg·min/L was specified by a regulatory agency, a wide range of disinfectant doses would yield satisfactory results: 100 mg/L with a contact time of 1 minute, 10 mg/L for 10 minutes, or 0.1 mg/L for 1000 minutes. However, if n for the specific microorganism was actually equal to 0.5, 316 minutes would be necessary for adequate disinfection at a concentration of 10 mg/L, rather than the 10 minutes necessary with n assumed to be 1. This underlies the hazards of relying upon generic indicators of disinfection efficiency.

As was discussed previously, first order disinfection kinetics are seldom followed, and tailing off curves frequently result. The CT model cannot account for this non-linearity when extrapolating data obtained at one level of inactivation to another. The 99% (2 logs) inactivation level has been used in most studies for determining CT values (Hoff 1987), and the validity of extrapolating to other levels of inactivation (1 log, 4 logs) is dependent on the nature of the inactivation curve from which the 99% inactivation data was determined. For inactivation of viruses, departures from first order kinetics usually occur only after 2 logs or more of the virus has been inactivated (Hiatt 1964). Similar trends exist for HPC bacteria (Wolfe et al. 1985). If inactivations in excess of 99% are required, and tailing-off kinetics exist, then the calculated CT value would be less than that actually required. Figure 3 illustrates such a situation.

Although caution must be exercised, the CT concept is applicable in certain situations. If the level of inactivation desired in the field is similar to that studied in the lab, and the kinetics are not affected by tailing off, CT values are a convenient way of regulating disinfection. For example, the CT values included in the SWTR for unfiltered surface waters (with

fewer than 1 *Giardia* cyst per 100 L of raw water) were selected to ensure removal of 99.9% (3 logs) of *Giardia lamblia* cysts, and 99.99% (4 logs) of viruses (U.S. Environmental Protection Agency 1989). These CT values were derived from experimental data for *Giardia,* that included inactivation levels greater than 99.9% (Hibler et al. 1987; U.S. Environmental Protection Agency 1989). In addition, for unfiltered sources, CT values to achieve 99.9% inactivation in the rule were set equal to the CT values necessary to achieve 99.99% inactivation under experimental conditions, to provide a margin of safety. As we have seen, however, this approach would be invalid if field disinfection was expected to inactivate 4 or 5 logs of cysts.

Because utilities are required to provide disinfection against the more resistant pathogen cysts and viruses, the resulting bacterial inactivation will be quite large, and most likely in the range of nonlinear kinetics. Due to the large extrapolation errors under these conditions, tabulated CT values are of little practical use for comparing the disinfection kinetics of bacterial species. Fitting the data to an accepted disinfection model would provide a superior descriptor of the kinetics.

3. MATERIALS AND METHODS

3.1. DESCRIPTION OF PILOT PLANT

The process configuration for the pilot plant used in conjunction with this study is illustrated in Figure 4. Raw water from the North Saskatchewan River was treated by conventional processes before pre-oxidation and one or more filtration operations. Stream 1 and Stream 5 were intended as control streams for comparison with the biologically treated streams (Streams 2 to 4). Stream 1 was prechlorinated to prevent biological growth in the filter media, and to provide a water without GAC adsorption treatment. Stream 5 represented a conventional treatment stream without any predisinfection. The filtration rates and filter media in Streams 2 to 4 were selected to investigate the effect of these parameters on biological treatment. It was the goal of this investigation to compare the disinfection response of biologically treated effluents to non-biologically treated waters. To this end, it was decided to compare two streams representing different degrees of biological treatment to a treatment process devoid of biological influences. Because of the application of chlorine prior to filtration, and the absence of a GAC contactor (which has been shown to be more readily colonized than anthracite/sand filters), Stream 1 was selected as a control stream to represent non-biological treatment. Streams 2 and 4 were selected to represent waters exposed to high rate and low rate of biological filtration, respectively. The inclusion of a GAC/sand filter employing PICA carbon, and the lower filtration rates used in Stream 4 were expected to produce larger biofilms than Stream 2.

Control Stream

Stream 1 effluent, sampled while the prechlorination system was not operated, was used to provide a control stream for comparison with the biologically treated streams. Samples were collected downstream of Filter 1 at least 4 hours after the prechlorination system was shut off. This provided a water treated by conventional means (coagulation, flocculation, dual media filtration), but without the presence of a biofilm on the filter media. It was necessary to suspend prechlorination prior to sampling for the disinfection experiments, since almost no HPC were present in the prechlorinated samples. The bacteria present in samples from this stream would therefore be representative of conventional treatment, but would not include biofilm organisms. Filter effluent was tested by the DPD colorimetric method (APHA AWWA WPCF 1989) prior to sample collection to ensure no detectable chlorine residual was present.

Other work done in conjunction with the biofilm thickness and density determinations at this pilot plant indicated that during the time of sampling, a small amount of biological growth was found on the Filter 1 media (Lu 1993, personal communication). Thus chlorination did not result in a sterile filter, although the biomass quantities were very small. This is consistent with findings by others that biological growth can occur in the presence of a chlorine residual (LeChevallier et al. 1992).

High Filtration Rate Biological Treatment Stream

Stream 2 effluent, sampled downstream of GAC Column 2 was used to represent a biologically treated stream with a high filtration rate.

Filtration rates through the anthracite/sand filter and the GAC contactor were twice that of Stream 4. Prior to experimentation, the ozone system at the pilot plant had been inoperative for a period of 6 weeks due to equipment malfunctions. Ozonation was restored two weeks before the first sample was taken from Columns 2 and 4. A biofilm measurement taken at that time indicated the presence of a 0.5 μm biofilm covering 50% of the GAC media in the first metre of the contactor.

Low Filtration Rate Biological Treatment Stream

The maximum biological treatment at the pilot plant was expected to occur in Stream 4. Filtration rates were half those in Stream 2, and a GAC/sand filter was used ahead of the GAC contactor rather than a dual media anthracite/sand filter. The lower flux rate of AOC through this stream, and the presence of the GAC/sand filter was expected to increase the biological activity in this stream. A biofilm measurement taken two weeks after ozone restart indicated the presence of a 0.3 μm biofilm covering 30% of the GAC media in the first metre of the contactor. This is less biological activity than exhibited in the high rate biological column as noted above. It should be noted that the biofilm measurements were only approximate, and therefore, during the early stages of ozonation, treatment was probably not as expected.

All samples for disinfection were collected in sterile containers, refrigerated at 4 °C, and analyzed within 24 hours.

3.2. DESCRIPTION OF BENCH SCALE APPARATUS

GAC Column

Prior to start up of the pilot plant, a bench scale GAC column was used to produce simulated biologically treated effluents, and provide a source of colonized GAC particles. Untreated North Saskatchewan River water, stored in a 5 gallon container, was circulated through two glass columns (plumbed in parallel) containing virgin GAC (Calgon F-400), at a rate of 300 mL/min by means of a peristaltic pump. Effluent from this apparatus was returned to the container serving as a raw water reservoir. Fresh river water was supplied bi-weekly to maintain an adequate supply of substrate.

PAC Reactors

In order to obtain suspensions with controlled amounts of GAC fines, and to ensure adequate HPC colonization time, several desorption experiments were conducted utilizing "PAC reactors". These reactors consisted of baffled, 2L beakers, each with two sample ports. An appropriate mass of GAC from the bench scale column was homogenized for 30s in 30 mL of bench scale column effluent to create fines approximately representative of those found in actual GAC effluents. This suspension was then added to 1L of North Saskatchewan river water and mixed for 3 to 6 days at room temperature, in the dark.

3.3. DISINFECTION PROTOCOL

HOCl and NH_2Cl Stock Solutions

Free chlorine stock solutions were prepared weekly to approximately 150 mg/L from Purified grade NaOCl (Fischer Scientific) in demand free water. Free chlorine residual was measured by the DPD colorimetric method according to Method 4500-Cl G (APHA AWWA WPCF 1989).

A preformed monochloramine stock solution of approximately 150 mg/L was prepared daily by mixing NaOCl and NH_2Cl solutions (prepared in pH 8.3 phosphate buffer) at a 3:1 Cl_2:N weight ratio (White 1972). The solution was mixed for 30 minutes, and the residual measured by the DPD colorimetric procedure (APHA AWWA WPCF 1989).

Demand Free Water And Glassware

Milli-Q water (Millipore Corporation) was shown to have a negligible chlorine demand, and was used in all instances requiring demand free water. Demand free glassware was prepared by exposing glassware to water containing at least 10 mg/L chlorine for 3 hours before use and rinsing with demand free water.

Procedure For Kinetic Experiments

The water sample to be disinfected was placed aseptically in a sterile, chlorine demand free erlynmeyer flask. One reaction vessel was used for each contact time. A separate vessel was used to monitor the degradation of the disinfectant residual. An appropriate volume of stock solution was then added to each vessel by sterile pipet. At appropriate time intervals,

sample was removed from the residual reactor, and the remaining disinfectant concentration was measured by the DPD method. After the desired contact time, disinfectant residuals were quenched by adding $Na_2S_2O_3$ (3 mL of a 0.1% solution used per 1L sample) to the reactor. Surviving HPC were then enumerated by membrane filtration. Experiments were conducted in random order. After enumeration, the morphology of the surviving HPC was noted for each time step in the experiment by observing the size and color of colonies, and their relative numbers. Experiments were conducted at room temperature (approximately 20 °C). Three replicates for each of Stream 1, Stream 2 and Stream 4 were conducted in this way.

3.4. MICROBIOLOGICAL METHODS

HPC Analyses

HPC bacteria were enumerated using the membrane filtration procedure according to Standard Method 9215D (APHA AWWA WPCF 1989). Samples from the first set of bench scale experiments were split and incubated at both 20°C for seven days and 35°C for three days on R2A agar (Difco Laboratories). Subsequent to this, all bacteria were incubated on R2A agar at 20°C for seven days.

Desorption Procedures

Attached HPC bacteria were desorbed by a physical/chemical procedure based on the work of Camper et al. (1985a) and optimized for this work, as will be discussed later. A Brinkman homogenizer with a PT10 generator probe (approximate tip diameter of 10 mm) was used for initial

experiments. Because approximately 100 mL of liquid was needed for homogenization with this probe, 90 mL of sample was mixed with 10 mL of concentrated desorption solution in a 250 mL erlynmeyer flask, to give a final concentration of desorption chemicals equal to that in the original method: 0.01 M Tris buffer at pH 7, 10^{-6} M Zwittergent 3-12, 10^{-3} M EGTA, and 0.01% peptone. The Tris buffer employed in the original method was not suitable for pH 5, so a phosphate buffer was used in all cases where pH 5 was required. Tris was used for all other cases. The flask containing the sample and desorption solution was surrounded by crushed ice during the homogenization procedure to prevent temperature increases. The original Camper method specified a homogenization rpm of 16000. In these experiments, rpm was approximated by setting the speed dial on the homogenizer to 7.5, based on the data contained in the Brinkman manual. Actual rpm level was not verified.

Midway through this research, it was discovered that the bushing on the Brinkman PT10 homogenizer probe was worn. For all subsequent desorption work, a Brinkman PT20 homogenizer probe (approximate tip diameter of 25 mm) was used in its place. Because this probe was larger, 500 - 700 mL of sample and the appropriate volume of concentrated desorption solution described above, was placed in a 1L erlynmeyer flask for homogenization. The homogenizer probe, and all equipment coming into contact with the sample to be analyzed, was sterilized by immersion in boiling water.

3.5. EXPERIMENTAL DESIGN

Significance Of Attached Bacteria

The goal of the desorption experiments was to develop an experimental method that could be applied to post-disinfected pilot plant effluents to determine if any bacteria attached to GAC fines which may be present in the effluent were penetrating the final disinfection barrier. To this end, a series of experiments was performed to determine if the Camper desorption technique (Camper et al. 1985a) was able to increase recovery of attached HPC bacteria in effluents characterized by low concentrations of carbon fines (on the order of 10µg/L). If the procedure could consistently increase recoveries in the presence of low numbers of fines that were known to be colonized, then subsequent results from applications of this technique to actual pilot plant effluents could be taken as a reliable indicator of the presence or absence of attached, viable HPC in the disinfected effluent.

Desorption methodology experiments

A screening experiment was conducted to determine the effect of modifying several procedural variables on the recovery of HPC bacteria. If any of the parameters were determined to be statistically significant for increasing bacterial counts, then the standard Camper homogenization technique would be modified accordingly. Because factorial designs can save much experimental effort in screening situations such as this, a 2^4 factorial design conducted as two half fractions (2^{4-1} resolution IV) was chosen (Box et al. 1978). The theory, applicability, and interpretation of

the results of this experimental design will be fully described in section 3.6. Three mid-point trials were performed to provided an independent estimate of the error mean square of the experiment. The experimental factors and their settings are tabulated in Table 4.

The bench scale GAC carbon column described in section 3.2 was used to provide a water sample containing GAC fines. In order to ensure the water had enough fines to provide large numbers of attached organisms, 2 g of GAC from the bench-scale column was homogenized at low speed to create fines, and returned to the column to supplement any fines that may have been present naturally. After mixing these fines with the media, flow was resumed through the column and the resulting effluent was collected for use in the desorption experiments. Each trial was treated in accordance with the conditions in Tables 4 and 5. HPC bacteria were then enumerated by the membrane filtration procedure. Fines isolated from several of the homogenized and non-homogenized samples were analyzed by Scanning Electron Microscopy (SEM) to determine the prevalence of attached bacteria.

Although several studies have concluded that HPC bacteria are recovered in the greatest number at lower temperatures and longer incubation times (Camper et al. 1985a), an experiment was designed to determine if time could be saved by reducing the incubation time to 3 days. Each trial from the factorial screening experiment was randomly split in two, with one half using R2A agar (Difco Laboratories) incubated at 20 °C for 7 days, and the other half incubated at 35 °C for 72 hours. Differences between the two growth conditions were determined using a paired t-test (Box et al. 1978).

Bench Scale desorption / disinfection experiments

As will be discussed fully in section 4.1, the results of the desorption methodology experiment indicated that a potential problem existed with applying the desorption technique to water samples containing low concentrations of carbon fines and high concentrations of non-attached bacteria. It was suspected that the number of bacteria desorbed from the carbon particles was not significant when compared to the large number of suspended organisms present in the sample. Therefore, experiments in the next phase were designed to determine if the application of the desorption technique would increase HPC recovery in effluents containing higher ratios of attached to suspended bacteria. Because attached bacteria are known to be quite resistant to disinfection, it was felt that by adding a disinfectant to the contents of the PAC reactors, suspended bacteria would be inactivated to a greater degree than the attached organisms. In addition, because the overall goal of these experiments was to investigate the efficiency of the homogenization technique at low concentrations of fines, PAC reactors for these disinfection/desorption experiments were prepared at varying carbon concentrations. It was suspected that the significance of any increases in HPC recovery by homogenization would decrease as the amount of carbon fines in the suspension decreased.

One sample in this series was not disinfected. This was done in order to verify that the failure of the desorption technique to increase bacterial recoveries was not due solely to an insufficient colonization period. Fines from this sample were again analyzed by SEM.

The experimental procedure was as follows. After sampling for initial HPC concentrations, sufficient chlorine stock was added to the PAC reactor described in section 3.2 to provide a free chlorine residual of approximately 2 mg/L. Samples were removed after contact times of 5 or 15 minutes, and analyzed for free chlorine. After the desired contact time, disinfectant residuals were quenched by adding $Na_2S_2O_3$ (3 mL of a 0.1% solution used per 1L sample) to the reactor. Samples were then either directly analyzed by membrane filtration, or homogenized before membrane filtration. In these experiments, an increase of greater than twofold in bacterial counts of homogenized over control samples was used as an indication of attached populations. This is consistent with the method of a similar study, in which this homogenization technique was used to desorb bacteria from carbon fines released from conventional GAC filter beds (Camper et al. 1986). No statistical basis for this criteria was provided in the original work.

Experiments at low fines concentrations

The next series of bench scale experiments was conducted to determine if the homogenization technique was capable of increasing the recovery of HPC bacteria when applied to water samples containing known amounts of carbon fines, present at the low concentrations expected in the pilot plant effluent. Before the homogenization technique can be used to enumerate attached bacteria in actual pilot plant samples with unknown carbon concentrations, it is necessary to show that the technique can increase HPC recoveries in samples known to contain the very low numbers of colonized fines expected in the pilot plant effluent. If it could

be shown that the desorption procedure would increase HPC recoveries when applied to samples with known concentrations of fines, then an increase in recovery with homogenization for a pilot plant sample could be taken to indicate the presence of an attached population. If no increase resulted, it would indicate that no significant viable, attached HPC bacteria were present in the pilot plant sample.

Several PAC reactors were prepared at a carbon fines concentration of 100 mg/L. HPC were first enumerated in these 100 mg/L samples by direct membrane filtration. Appropriate volumes of liquid were taken from these reactors and diluted into autoclaved pilot plant effluent to obtain suspensions with low fines concentrations (10 mg/L to 10 µg/L). These new suspensions were then homogenized with the PT10 probe prior to enumeration by membrane filtration. In the first experiment of this type, the homogenized recoveries were compared to the expected non-homogenized recovery (obtained by multiplying the HPC density in the 100 mg/L non-homogenized PAC reactor by the appropriate dilution factor). This procedure was repeated with the contents of the PAC reactor first being exposed to 2.5 mg/L of free chlorine for 30 minutes. It was following these experiments that the damage to the PT10 probe described in section 3.4 was discovered. All subsequent desorption work was done with the PT20 Probe.

In order to replicate above experiments, and to verify that results obtained with the PT20 probe were comparable to those previously obtained with the damaged PT10 probe, the non-disinfected dilution experiment was repeated. In this experiment, each dilution sample was enumerated by direct membrane filtration and membrane filtration with

prior homogenization, rather than using calculated values for the non-homogenized samples.

Pilot Scale desorption experiments

As it was determined that the Camper technique could successfully recover attached bacteria from colonized GAC fines present in concentrations as low as 10 μg/L, this procedure could be legitimately applied to actual pilot plant effluent to evaluate the impact of fines on the final treated water quality. To assess the presence of viable, attached bacteria penetrating the final disinfection barrier, the homogenization technique was applied to pilot plant effluents after exposure to free chlorine and monochloramine. As before, ratios of homogenized to non-homogenized recoveries greater than two were taken to indicate the presence of significant attached HPC populations on the surface of released GAC fines.

The pilot plant samples from the streams described in section 3.1 were disinfected by exposure to an average residual of about 0.5 mg/L free chlorine for 30 minutes, or to about 5 mg/L preformed monochloramine for 120 minutes according to the protocol of section 3.3. Disinfectant residuals were found to be effectively constant for all experiments. These doses were chosen to reflect disinfection conditions expected in practice. After disinfection, samples were split and analyzed by direct membrane filtration or membrane filtration with prior homogenization.

Disinfection Kinetics

The objective of the disinfection kinetic experiments was to determine if any differences existed in the disinfection response of biologically and non-biologically treated effluents. The disinfection kinetics of each stream were compared by studying the inactivation of HPC bacteria as a function of time, at a constant disinfectant concentration. Data collected in these experiments was fitted by non-linear regression techniques to a simplified version of the Hom model, which can be represented as follows:

$$\log N/N_o = -kt^m$$

where N/No is the ratio of the surviving organisms at any time to the initial number; k is a constant incorporating both the reaction rate constant and the disinfectant concentration; t is time; and m is an empirical constant which accounts for variations in the inactivation rate with time. The different streams were compared on the basis of the model parameters k and m.

Because the objective of this research was to compare the kinetics of several streams under conditions indicative of water treatment practice, the Hom model was simplified to allow the use of a single disinfectant concentration. Any differences found between the biological and non-biological streams could be tested at other concentrations in the future to confirm behaviour under different conditions.

Disinfectant concentrations and contact times were selected to represent the disinfection behavior under conditions representative of full scale

operation. Free chlorine experiments were conducted within a concentration range of 0.35 to 0.47 mg/L, and at contact times up to 30 minutes. Concentrations of monochloramine varied between 3.99 and 5.18 mg/L, with a contact time of 120 minutes. This range of actual disinfectant concentrations was considered to represent a constant residual for these experiments. It was felt that over such a concentration range, variations in the HPC population and water quality parameters would exert a greater influence on the inactivation rate than these small changes in the disinfectant concentration between experiments.

3.6. STATISTICAL ANALYSES

Factorial Experiments For Desorption Methodology

Numerical analysis of the screening experiment for desorption procedures was conducted according to a factorial experimental design (Box and Hunter 1961; Box et al. 1978; Davies 1979). In the desorption methodology experiment, it was desired to estimate the significance of 4 parameters, using two different levels of each variable. This would normally correspond to a 2^4 factorial requiring 16 trials. In order to reduce experimental effort, a 2^{4-1} resolution IV design was chosen (Box and Hunter 1961). The design matrix was established by setting the column for the 4th variable to the value for the 123 interaction, i.e. a defining relationship of I=1234. In such a resolution IV design, no main effect is confounded with any other main effect or two factor interaction. However, the two factor interactions are confounded with each other in the half fraction. If analysis reveals that the confounded interactions may be significant, the complementary half fraction may be performed, and

the effect of each interaction can be computed separately. The design matrix and confounding pattern for this factorial are shown in Tables 5 and 6. Variables are coded such that the upper level of the variable is given the notation +1, while the lower level is -1. Midpoint settings are coded as zero.

Because all of the trials could not be completed at once, and the composition of the effluent / fines suspension was not constant from day to day, the design was arranged in 4 blocks of two experiments each, performed in random order. Block factors were set to the interactions of 12 and 23 (Box and Hunter 1961). The mean square of the pure error calculated from three replicates conducted at the variable midpoints was used as an estimate of the variance. Based on this variance, the 95% confidence interval (CI) for each of the calculated effects was determined using a two sided t-test. An effect was considered to be significant if its 95% CI did not contain zero.

As a further check, a half normal plot was used to suggest which effects might be significant. In such a plot, the abscissa is the absolute value of the calculated effect, and the ordinate is the probability, P_i, given by:

$$P_i = (i - \tfrac{1}{2}) / n, \quad i = 1,2,\ldots,n.$$

In the case of a null experiment, the resulting line would be straight when plotted on probability paper. The spread of the points about the line would reflect the degree of precision. If any of the effects were statistically significant, the resulting line would be nonlinear, with the deviating points representing significant effects.

Non-Linear Regression Of Disinfection Data

The results of the disinfection kinetics experiments were fitted to the simplified kinetic model by nonlinear least squares techniques (Box et al. 1978; Draper and Smith 1981). The parameter estimates yielding the minimum residual sum of squares (SS) were determined using a quasi-Newton search technique.

An estimate of the joint confidence region for the parameter estimates was used to determine which combinations of the three treatment streams were statistically different and which were similar. The residual SS corresponding to the 95% joint confidence region (JCR) was calculated for each model and data set, based on the following formula (Box et al. 1978):

$$SS(\beta)_{.95} = SS(\beta)[1+\frac{p}{n-p}F_{.95(p,n-p)}]$$

in which $SS(\beta)_{.95}$ is the critical level of the sum of squares; $SS(\beta)$ is the minimum sum of squares; p is the number of estimated parameters; n is the number of observations; and $F_{.95(p,n-p)}$ is the upper 5% point on the F distribution with p and n-p degrees of freedom. This linear approximation to the true non-linear confidence region is based on the assumption that the vector of parameter estimates is normally distributed, which is not the case for the non-linear situation. This approximation gives the correct shape of the confidence region, but at a slightly incorrect probability level. Any set of parameters that results in a SS less than the critical value is considered to be included in the 95% JCR, and therefore a statistically valid parameter estimate.

The JCR for each treatment stream and disinfectant was generated using the non-linear estimation techniques described above, to generate the parameter estimates corresponding to the critical level of the sum of squares. Graphical representations of these JCRs were used to compare the statistical similarity of the biological and non-biological treatment streams at the pilot plant. Treatment streams with overlapping confidence intervals, i.e. those which contain a common subset of parameter estimates, were considered statistically similar at the stated confidence level.

Statistical differences in the overall inactivation levels for each stream were determined by examining the confidence interval associated with the predicted values. These confidence limits were calculated using the t statistic at the 5% and 10% significance level. The mean square of the residual (the residual sum of squares divided by the degrees of freedom) was used as an estimate of the model variance (Box et al. 1978).

4. RESULTS AND DISCUSSION

4.1. ATTACHED HPC BACTERIA

Desorption Methodology

The results of the bench scale methodology experiment revealed that none of the desorption procedural variables investigated were found to significantly increase or decrease HPC recoveries, even though large numbers of fines were present (samples were visibly black). Parameter confidence intervals showed that none of the estimated effects were significantly different from zero at the 5% significance level. Results from the half normal plot also indicated that there were no statistically significant factors. A detailed summary of these calculations is included in Appendix A.

SEM analysis revealed that very few colonized particles were present in both the homogenized and non-homogenized samples. As well, particles appeared to have either regions of heavy colonization, or no visible bacteria at all. Although only limited analysis was performed, the colonized regions of the non-homogenized fines appeared to contain more bacteria.

The failure of homogenization to increase HPC recoveries led to two conclusions. First, the numbers of suspended bacteria in the bench scale effluent ($\approx 10^4$ CFU/mL) may have masked the presence of any attached organisms liberated in the desorption treatment. This was confirmed in subsequent experiments. Secondly, the carbon fines present in the bench scale experiment may not have been adequately colonized before homogenization. The fines created by crushing were only in contact with

the effluent for a short time before treatment. Particles that did exhibit attached bacteria may have originated on an exposed surface of the intact GAC granule before crushing. However, because variations in technique did not increase recoveries, and because the original Camper method has been extensively applied to GAC particles, it was decided to use the standard Camper method for the remainder of the study.

Data from the bench scale screening experiment was also used to determine the best incubation temperature and time for the recovery of HPC bacteria. Analysis by a paired t-test (Box et al. 1978) indicated that HPC recoveries from plates incubated at 20°C for seven days were significantly greater than those incubated at 35°C for 72 hours at the 5% significance level. For this reason, all subsequent experiments were conducted at 20°C for 7 days.

Effect of Background Un-Attached Bacterial Populations

The results of the desorption/disinfection experiments are summarized in Table 7. Significant attached populations were recovered in all of the disinfected samples. Only the nondisinfected sample did not exhibit an increase in recovery greater than two. This confirmed that insufficient colonization time was not the only explanation for the failure of the desorption technique to increase HPC recoveries in the initial methodology experiments.

By subtracting the homogenized recoveries from the untreated recoveries, an estimate of the attached bacteria can be made. Results for the disinfected samples indicate that the attached bacteria were in the order of 10^4 CFU/mL. This explains why increases in recovery were not seen in

the nondisinfected solution and the bench scale column, both of which supported suspended HPC populations in the order of 10^6 CFU/mL. In our solutions with 10 to 100 mg/L of carbon, this represents approximately 10^8 to 10^9 viable organisms per gram of carbon. When compared to the biofilm densities reported in the literature for intact GAC granules (Table 1), these numbers appear to be rather high. However, it should be noted that the surface area available for colonization is much greater on an equal mass of crushed GAC compared with grains of filter media. The data in Table 7 also illustrate that as the concentration of carbon fines decreases, the effect of homogenization also decreases, as would be expected. In general, exposure to 2 mg/L chlorine for 5 and 15 minutes resulted in approximately 1 to 2 logs reduction. At these high carbon concentrations, a significant chlorine demand was exerted by the GAC fines.

SEM analysis of the nondisinfected sample in this series of experiments revealed that many of the particles present showed no evidence of colonization. The absolute numbers of sterile particles in these experiments compared to the previous ones could not be determined. Again, regions with attached bacteria showed the greatest density in the non-homogenized samples.

Efficiency of Desorption Technique at Low Concentrations of Fines

The results gathered from the GAC fines dilution series indicated that the homogenization technique was able to increase bacterial recovery compared to direct enumeration, in water samples containing 100 mg/L to 10 µg/L of colonized carbon fines. Tables 8 through 10 and Figures 5

through 7 document the increases in HPC recovery when samples were homogenized prior to enumeration for both disinfected and non-disinfected samples. By comparing the recovery increases in the non-disinfected samples to the increases for the disinfected sample, it can be seen that the efficiency of the homogenization technique is greater when the ratio of attached bacteria to suspended bacteria is increased, as was demonstrated in the previous experiments. For example, at 1 mg/L of fines, the ratio of homogenized to non-homogenized HPC recoveries was found to be 1.90 and 4.01 for the two non-disinfected samples (Tables 8 and 10). These samples contained suspended HPC concentrations of 3160 and 4600 CFU/mL respectively. However, in the disinfected sample with a suspended population of < 1 CFU/mL and the same concentration of fines, the application of the homogenization technique increased recovery by 49 times (Table 9).

A comparison the results in Tables 8 and 9, which were obtained from the same PAC reactor, indicate that exposure of the reactor contents to 4.5 mg/L FAC for 30 minutes inactivated approximately 4 logs of unattached HPC, but less than 2 logs of attached bacteria. These results are similar to those obtained previously with the PAC reactors and 100 mg/L of carbon fines. A significant chlorine demand was again noticed at this concentration of carbon fines.

The results of the non-disinfected experiment conducted with the PT10 probe (Figure 5) appear to suggest that at very low fines concentrations, increases in recovery via homogenization are greater than at higher fines concentrations. The suspensions of various fines concentrations used in this experiment were obtained by pipetting an appropriate amount of the

PAC reactor contents into autoclaved pilot plant effluent. Because of the small transfer volumes involved, successive dilutions were obtained from previous dilutions, rather than directly from the original PAC reactor at lower fines levels. When attempting to extract a small volume of water containing 10 µg/L of carbon (3 or 4 fines) from a one litre reaction vessel, accuracy can be lost, since a fine may be either present or absent in the transfer pipet. Therefore, the increased recovery at 10 µg/L of carbon may be due to a greater number of fines being present in this suspension due to dilution errors. Secondly, the non-homogenized recoveries shown in Table 8 and Figure 5 were calculated from the results obtained from the 100 mg/L suspension. If each sample had been truly analyzed both with homogenization and without, dilution errors may have shown up.

The most likely explanation for the results obtained with the PT10 probe is deficiencies in the probe itself. During these experiments, it was noticed that samples homogenized by this probe contained more unidentified black particles than were expected in suspensions of low GAC concentration. Subsequent examination of the probe revealed that the bushing was worn, and metal filings were being released into the homogenized solutions. The effect of these filings was most noticeable in solutions that were relatively carbon free. It is not known for how long previous to this metal filings were being released into the samples, as the carbon concentrations analyzed were much higher, and homogenization always resulted in blackening of the samples.

The results summarized in Table 9 and Figure 6 for disinfected suspensions were also obtained with the PT10 probe. Although metallic particles were seen on the membrane filters at higher sample volumes, the

results do not appear to be affected. A comparison of Tables 8 and 10 indicates that the PT20 probe can be considered equivalent to the PT10 probe, since increases in recovery were similar for most carbon concentrations under similar conditions. The differences in recovery obtained for the 0.01 mg/L fines concentration was likely due to experimental abberations, rather than to differences in the performance of the two probes.

The results obtained with the PT20 probe on non-disinfected samples summarized in Table 10 and Figure 7 are the most useful for assessing the applicability of the Camper desorption technique to increasing recovery of HPC bacteria in the presence of low concentrations of colonized carbon fines. These results indicate that at fines concentrations as low as 10 μg/L, increases in recovery greater than two were realized after homogenization. These increases were obtained in the presence of suspended HPC populations of 25 CFU/mL. Application of this procedure to post-disinfected pilot plant effluents expected to contain 10 μg/L of carbon but much lower suspended HPC concentrations (<1 CFU/mL for post-chlorination, <10 CFU/mL for post-chloramination), would be expected to show even greater increases in recovery if significant numbers of viable, attached bacteria were present.

Estimates of Bacterial Density on GAC Fines

By subtracting the non-homogenized recoveries from the homogenized recoveries, the number of attached HPC desorbed from the carbon fines can be estimated. Table 11 summarizes the observed densities of HPC bacteria attached to GAC fines collected during this research.

Because SEM analysis indicated that some bacteria remain attached to the fines after being subjected to the desorption procedure, these tabulated densities most likely underestimate the actual degree of colonization. The number of attached HPC recovered from suspensions of non-disinfected GAC fines ranged from 10^9 to 10^{10} CFU/g of carbon, while disinfected samples exhibited 10^7 to 10^9 CFU/g.

Literature estimates of the number of bacteria attached to GAC media particles were reported as varying between 10^4 to 10^8 CFU/g of carbon (Table 1). Our results for GAC fines are substantially higher. One reason for the higher densities seen on our GAC fines, is that the surface area of crushed GAC is much larger than that of intact GAC granules. This corresponds to a greater number of surface sites for attachment. As well, the fines examined in this study were allowed to colonize for 3 to 6 days, in the absence of hydraulic shear forces, oxidizing substances, or extreme environmental conditions. Growth conditions for the attached organisms isolated from the GAC beds of operating water treatment plants may be less favorable.

Recovery of Attached HPC Populations in Pilot Plant Effluents

From the above results, it can be concluded that if colonized GAC fines with viable HPC penetrate the disinfection barrier, the application of the homogenization technique to the effluent should result in increased recoveries of HPC bacteria when compared to the same effluent enumerated by direct membrane filtration. No increase in recovery can be assumed to indicate that no viable attached HPC are present. Table 12 summarizes the results of applying the desorption technique to

disinfected pilot plant effluent. None of the samples showed an increase in recovery by a factor of greater than two when homogenized. One homogenization experiment was also conducted on a nondisinfected effluent sample taken from Stream 2 of the pilot plant. The homogenization technique did not appreciably increase HPC recoveries on this non-disinfected effluent sample either. From these results, it was concluded that significant numbers of attached bacteria were not penetrating the disinfection barrier by attachment to released GAC fines.

Significance of Desorption Results

Based on estimates of the expected number of bacteria attached to GAC fines found in the literature (Table 1), the presence of attached organisms is insignificant when compared to the numbers of suspended bacteria. In particular, based on an assumed biofilm density of 10^6 CFU/g of carbon, and effluent fines concentrations of 10 μg/L, only 10 CFU/L (0.01 CFU/mL) of attached bacteria would be expected in typical GAC effluents (DiGiano et al. 1990). When compared to the number of surviving, unattached bacteria found after extended disinfection contact times in this research (<1 CFU/mL for post-chlorination, <10 CFU/mL for post-chloramination), 0.01 CFU/mL of attached bacteria would not be significant. Even if the upper range of reported values (434 CFU/L) is considered (Stewart et al. 1988), the attached bacteria may not be significant after a reasonable disinfection contact time.

The results presented here suggest that biofilm densities on GAC fines may be as high as 10^{10} CFU/g. Even disinfected fines maintained densities of 10^7 to 10^9 CFU/g which for an effluent with 10 μg/L of fines,

becomes 0.1 to 10 CFU/mL of disinfected effluent. These levels of colonization would be expected to show up in the post-disinfected effluent samples investigated in this research after homogenization, since after 30 minutes of chlorination, HPC were often less than 0.1 CFU/mL. However, the application of the homogenization technique did not significantly increase HPC recovery in pilot plant effluent disinfected with free chlorine or chloramines. It cannot be concluded from these results whether this is because there were insufficient numbers of fines being released, if the fines being released are not colonized to a great extent, or if the attached bacteria are being inactivated by final disinfection.

As a fines monitoring program was not included in the scope of early work at the pilot plant, the number of GAC fines being released in the effluent is unknown. In addition, the GAC columns were backwashed weekly. The impact of backwash on fines release was not investigated. GAC fines created by interparticle friction during backwash may be removed in the wash water, or may not have sufficient time between backwashes to pass through the bed and into the effluent.

The ozone system was not fully operational for a long period of time before the desorption experiments were performed. Because of this, attached bacterial populations on any released fines could have, potentially, been lower than expected. Biofilm analysis of the GAC media indicated that only 50% of the media surface in contactor 2 and 35% in contactor 4, was covered in biofilm (Lu 1993, personal communication). If the numbers of attached bacteria were closer to 10^6 CFU/g as proposed in

the literature rather than the higher densities found in this study, homogenization would not be expected to increase recoveries.

A third explanation for the apparent lack of attached bacteria in the disinfected pilot plant samples is that the attached HPC may have been inactivated in the disinfection process. Exposure of suspensions containing 100 mg/L of crushed GAC to 2 to 5 mg/L of FAC for 30 minutes resulted in inactivation of attached organisms in the order of 1 to 2 logs. However, disinfection of bacteria attached to intact GAC media particles with 2.0 mg/L FAC for one hour has been reported to result in no inactivation (LeChevallier et al. 1984). Others have concluded that GAC fines may not be difficult to disinfect if they are present in the concentrations expected under normal operating conditions (DiGiano et al. 1990). Our results may therefore be further evidence that released GAC fines are not a threat to public health if normal disinfection procedures are in place.

4.2. DISINFECTION

Results from the kinetic experiments for both free chlorine and monochloramine indicated that the disinfection kinetics of biologically treated and non-biologically treated streams were statistically different. However, the overall levels of inactivation predicted by the kinetic models were statistically similar (α=0.05) for both the biologically and non-biologically treated streams, for the same disinfectant. With free chlorine as the disinfectant, the kinetic parameters for all three streams examined were statistically different from each other. However, with monochloramine, although the kinetics of the two biological streams were

statistically different than the control stream, they were not statistically different from each other. The kinetics for all streams with both disinfectants were non-linear, with decreasing rates of inactivation seen at extended contact times.

Kinetic Modeling

The results of the kinetics experiments are shown in Figures 8 through 13. The non-linear least squares parameter estimates and the residual sum of squares for the simplified kinetic model are summarized in Table 13, and represented graphically in Figures 14 and 15.

FAC

Figure 14 shows the manner in which the inactivation kinetics of the treatment streams differ for disinfection with free chlorine. As can be seen, the initial rate of inactivation was greatest for Filter 1, the conventionally treated control stream. During the first few minutes of contact time, Column 4, the stream with maximum biological treatment, was on average the most difficult of the three to disinfect. At longer contact times, little additional inactivation is seen for Filter 1, while the HPC populations in the biological streams continue to decrease slightly. In general, all three kinetic curves exhibit a similar shape and a marked non-linearity. After 30 minutes contact time, all three streams showed inactivations of approximately 4.0 to 4.5 logs.

Filter 1 has the largest reaction constant, k, of the three test streams. This corresponds to a steeper initial slope and quicker inactivation of the main part of the HPC population. The m parameter was very small for this

stream, resulting in a very sharp bend in the inactivation curve, and the occurrence of little additional inactivation after 5 minutes.

Columns 2 and 4, although not statistically similar, are qualitatively similar. The k constant for these streams is less than that for the control stream, indicating that in general, the HPC contained in the effluent are more resistant to free chlorine than those in Filter 1. The k for Column 2 was slightly greater than that of Column 4, perhaps reflecting the degree of biological treatment each received. The m values for these streams were significantly larger than the control stream. The consequence of this is a lesser degree of curvature in the tailing off effect, as can be seen in Figure 14.

CAC

The variations in disinfection response with monochloramine are shown in Figure 15. At extended contact times, Filter 1, the conventionally treated control stream, exhibited a greater tailing-off effect, and slightly lower average inactivation than the biological streams. Column 4, the stream with maximum biological treatment, had the greatest average predicted inactivations. At contact times less than 30 minutes, the kinetic behavior is reasonably similar for all effluents, although the control stream exhibits a slightly greater initial inactivation rate. The differences during the early stages of disinfection are not, however, as distinct as for the FAC results. Again, tailing off occurred at extended contact times, although the resulting curvatures were less than was observed with free chlorine. After 60 minutes contact time with a monochloramine residual of roughly 5.0 mg/L, all three streams would be expected to show an

inactivation of 3.0 logs. As was the case for FAC disinfection, Filter 1 showed the largest k constant, and the smallest m value of the three test streams. However, m values in general for NH_2Cl were much higher than those computed for the free chlorine streams.

Statistical independence of treatment streams

The critical sum of squares value corresponding to the border of the 95% joint confidence region of the simplified kinetic model was calculated for each stream for both disinfectants. The resulting JCRs are shown graphically in Figures 16 and 17 for free chlorine and monochloramine respectively. As can be seen in Figure 16, none of the joint confidence regions overlap. Therefore, at the 95% confidence level, the free chlorine disinfection kinetics of all treatment streams were significantly different. Figure 17 indicates that for disinfection with preformed chloramines, the kinetics of both biologically treated streams were significantly different than the control stream, but were not significantly different from each other.

Calculation of the 95% confidence intervals for the predicted inactivation levels indicated that the overall disinfection resistances of the biologically and non-biologically treated streams were not statistically different. At all contact times, the 95% confidence intervals for the predicted inactivation levels overlapped for all streams (calculations are included in Appendix B). However, at a 10% level of significance and contact times less than 1 minute, Filter 1 exhibited statistically greater inactivations than Column 4. Similarly, at a contact time of 120 minutes, Column 4 exhibited statistically greater inactivations than Filter 1 for monochloramine

disinfection. Column 2 effluent was not significantly different at either significance level. Because any significant differences in overall disinfection resistance were limited to the extreme ends of the data collected, and only occurred under reduced levels of significance, it was concluded that the biologically and non-biologically treated streams exhibited similar overall resistance to both disinfectants.

Morphology of Surviving HPC

Organisms in the Filter 1 effluent prior to free chlorine disinfection were of no predominant type. Uniform, round colonies of light yellow, white, red, dark green and orange were present. Only on one occasion was a small number of pink colonies observed from the control stream samples.

HPC found in the effluent of Columns 2 and 4 were predominantly white, yellow and light green. Small numbers of pink colonies were observed in all of the effluent samples for the ozonated streams. The larger variety in organisms observed in the control stream was not found.

FAC

Between 30 seconds and 5 minutes of exposure to FAC, the colonies recovered from the Filter 1 effluent were primarily white, light green, and yellow. After 30 minutes, only white and light green colonies were present. No pink colonies were observed in any of the samples disinfected with free chlorine.

The morphology for the chlorinated samples from Columns 2 and 4 were found to be similar to each other. As the contact time increased, the number of pink colonies recovered from the biologically treated samples

increased, until between 5 and 30 minutes of contact, the plates usually consisted of equal numbers of pink, white, light green and yellow colonies. In contrast to the Filter 1 data, pink colonies predominated after 30 minutes of exposure in several samples.

CAC

For all contact times with combined chlorine, the predominant bacteria recovered from the Filter 1 effluent were very small white, yellow and light green colonies. A pink colony was seen only once in the CAC data for Filter 1, corresponding to the experiment in which pink colonies were observed in the pre-disinfection sample.

No specific trends were observed for the HPC recovered from Columns 2 and 4 after CAC application. The predominant colonies after disinfection were yellow, white, and colorless. Some pink colonies were still observed after 5 minutes of contact time, although none appeared at longer contact times.

Discussion

Non-linearity

Our morphology observations indicate that the tailing off effect seen in the free chlorine data may be due to the presence of a resistant subgroup within the HPC population. After 30 minutes of contact time, all of the colonies for Filter 1 were white or light green. For the biologically treated streams, the number of pink colonies increased from relatively few initially, to being the predominant organism after disinfection. In a study by Wolfe (1985), identification of the bacteria surviving exposure to FAC

were qualitatively similar to these observations (Wolfe et al. 1985). Before disinfection, 47.2% of the HPC were identified as from the genus *Pseudomonas*, while only 10.0% were *Flavobacterium*. After 1 minute of contact time at 0.75 mg/L FAC, the distribution was 8.0 and 88% respectively. After 30 minutes, all isolates were red pigmented *Flavobacterium*. Significantly, the levels of these chlorine tolerant bacteria (2900 CFU/mL) did not decrease between 30 and 60 minutes of exposure. This again parallels our study. Red pigmented *Flavobacterium* were also found to be the predominant species to survive chlorination in a separate study involving a GAC pilot plant using predisinfection with chlorine dioxide (Stewart et al. 1988). Although bacteria from our study were not identified, the rapid inactivation of the majority of the subgroups, followed by a decreasing rate of inactivation for the remaining chlorine resistant group was similar to these studies. In addition, the predominant chlorine resistant subgroups seen in the biologically treated samples was of red (pink) pigmentation.

The presence of a single resistant subgroup in the monochloramine population was not seen in our data. Two or three different types of colonies were observed at the end of the contact time. In the study by Wolfe, monochloramine survivors consisted of members of several genera. A predominant group was not always present. However, in other studies (Stewart et al. 1988), GAC column effluent samples exposed to chloramines developed bacterial populations that were exclusively white pigmented *Pseudomonas* spp.

For free chlorine, the control stream displayed the most pronounced kink in the kinetic curve, the value of m being half that for the chlorinated

biological streams, and a full order of magnitude less than that for the CAC experiments. In addition, the morphological observations indicated the presence of a single group of white and light green HPC at contact times greater than 5 minutes. These results suggest that the decreases in the rate of inactivation may be attributed to two discrete subpopulations of HPC; one of minimal resistance characterized by rapid initial inactivation ($k=5.52\ min^{-1}$) and one which is highly chlorine tolerant. The smoother curve (higher value of m) for the biologically treated streams and the lack of a single morphology at extended contact times suggests that more than one resistant subgroup was present in the HPC population after ozonation. This is in contrast to the single resistant subgroup observed with the control stream.

The values of m for the CAC data were larger than those for FAC. As well, morphology observations from this study and others (Wolfe et al. 1985), indicate that chloramination survivors are members of more than one genus. The kinetics curves for monochloramine did not become flat as those for FAC did. Even after 120 minutes of contact time, the rate of inactivation was not zero. This implies that monochloramine may be less selective than FAC, and especially effective against the chlorine resistant, pink colored subgroup seen in this study.

Comparison of biological and control streams

The differences between the disinfection kinetics for the biologically treated streams and the control stream can be attributed to differences in the post-filtration HPC population, and the variations in biocidal effectiveness between free chlorine and monochloramine.

For disinfection with free chlorine, the biologically treated streams exhibited a lower rate of inactivation than the control stream at shorter contact times. This can be explained by considering the effects of preozonation on the HPC population. After ozonation, any given surviving bacteria may attach to the GAC media, multiply, and later be removed in the effluent after sloughing from the biofilm. Alternately, the bacteria may be immediately removed in the effluent without attachment. It has been shown that prior disinfectant exposure may influence sensitivity to disinfection (Carson et al. 1972), and result in the selection of resistant survivors. HPC bacteria in distribution system samples were found to be more chlorine resistant if they had come from chlorinated water supplies as compared to those from unchlorinated supplies (Ridgeway and Olson 1982). Because ozone is such a potent biocide, only the most resistant organisms will survive to colonize the GAC columns. It is these resistant bacteria that multiply within the system and make up the HPC population in the column effluents. Their inherent resistance, and perhaps their increased resistance due to prior disinfectant exposure, make them more difficult to inactivate than the HPC found in the control stream. The majority of the easily disinfected bacteria found in the control stream are removed by ozonation prior to exposure to chlorine. The pink colonies recovered in the biological effluents are most likely members of this ozone and chlorine resistant subgroup. Because a greater proportion of the HPC present in the biologically treated effluents are from this resistant subgroup, the biologically treated streams were slightly more difficult to disinfect than the non-ozonated stream at short contact times. It must be emphasized that, as shown in the previous

section, increases in disinfection resistance were not due to the release of colonized GAC fines, and that overall resistances were the same for all streams regardless of the degree of biological treatment each received.

For disinfection with monochloramine, the results suggest that the biological streams are more amenable to disinfection than the control stream, due to the lesser degree of tailing-off seen at extended contact times. Monochloramine has been shown to result in comparable or greater inactivations than FAC in biologically treated effluents (LeChevallier et al. 1992; Stewart et al. 1988; Wolfe et al. 1985). The primary reason for this appears to be that although some bacterial groups such as *Flavobacterium* are exceptionally resistant to disinfection by FAC, they do not exhibit the same level of resistance against monochloramine. As was seen in our study, the pink bacteria present at extended contact times with FAC were successfully inactivated by CAC. This trend was also seen for the FAC resistant *Flavobacterium* found in other studies (Stewart et al. 1988)(Wolfe et al. 1985). It is also possible that monochloramine resistant HPC present in the natural water population were successfully inactivated during preoxidation. The absence of these organisms would then explain the increased performance of CAC for biologically treated effluents compared to the control stream. Sufficient evidence is not available from this study to verify this hypothesis.

The ability of preformed monochloramine to better disinfect biofilm organisms has also been documented (LeChevallier et al. 1988). This was believed to be a result of the greater penetrating power of combined chlorine compared to free chlorine. Although the presence of attached bacteria was shown to be insignificant in this study, the affinity of CAC

for biofilm organisms may have been a factor in the greater inactivations seen with the biological streams. Data from the pilot plant indicated that very few native HPC bacteria survived the preoxidation step. The low numbers that did penetrate into the filters were insufficient to be solely responsible for the HPC levels found in the effluent. Therefore, the majority of the effluent bacteria must have been organisms that had multiplied on the filter media and were subsequently sloughed from the biofilm. If these organisms were still covered in extracellular slime, or attached to each other or other detritus, the greater penetrating power of NH_2Cl may have been responsible for their efficient removal.

Practical implications of results

Overall inactivation rates were greater with free chlorine than preformed monochloramine on a weight for weight basis for similar contact times. Approximately 3.5 to 4 log units of HPC were inactivated with a FAC residual of 0.35 - 0.45 mg/L in less than 5 minutes for all streams. With monochloramine, it required 30 minutes of exposure to approximately 5 mg/L to inactivate 3 log units. However, a comparison of Figures 14 and 15, indicates that 4 logs inactivation was achieved for biological effluents exposed to 0.5 mg/L of free chlorine for 30 minutes and 5 mg/L of preformed monochloramine for 60 minutes. Although free chlorine is a much faster biocide than monochloramine the tailing-off effect resulted in little additional kill after the first 5 minutes. After 120 minutes, the higher concentration of CAC resulted in greater inactivations than FAC for the biological streams. Because post-disinfection with chloramines produces fewer THMs than post-chlorination, and because NH_2Cl residuals are often required to provide a stable disinfection residual in the distribution

system, final disinfection with monochloramine may be superior for biological treatment installations able to provide long detention times.

In general, overall inactivations were similar for the biologically and non-biologically treated waters. Therefore, no adverse impacts on disinfection are anticipated if BAC processes are implemented in full scale installations. However, since biologically treated effluents disinfected with FAC were found to have lower inactivation rates than the conventionally treated stream at short contact times, conventional treatment plants employing post-chlorination considering conversion to biological treatment should carefully monitor disinfection performance. Decreases in disinfection efficiency may not occur if the source water does not contain a subgroup of organisms with a high FAC resistance.

Post-chloramination as applied in the field (pre-ammoniation or concurrent addition) may provide the best overall disinfection performance for BAC installations. The presence of transient free chlorine prior to complete monochloramine formation, and the possible synergistic effects of free chlorine and monochloramine together, often result in inactivations in the field being of a similar magnitude to those resulting from free chlorine alone (Kouame and Haas 1991; Ward et al. 1984; Wolfe et al. 1984). Final disinfection in this manner would exhibit the rapid initial inactivations typical of free chlorine, but also have the ability to destroy the resistant subgroups of the population with chloramines. Trihalomethane formation is not expected to increase compared to preformed chloramination. Through reactions with ammonia, little or no free chlorine would be available for reaction with the THM precursors since although the DBP reaction is generally considered to be quick, it is

relatively slower than the formation of chloramine under optimum conditions (Berman et al. 1992). However, because the rate of formation and the biocidal activity of monochloramine are very pH dependent, this process scheme may not be optimum for all waters.

5. CONCLUSIONS

Investigations into the applicability of the Camper desorption technique led to the conclusion that the procedure was capable of recovering HPC bacteria attached to GAC fines in the disinfected pilot plant effluent. It was found that the desorption technique was successful in recovering attached bacteria from suspensions containing as few as 10 µg/L of fines. However, the procedure was not effective in recovering attached bacteria from low numbers of fines if a significant population of unattached bacteria was present, as would be the case for non-disinfected effluents. GAC fines colonized in the lab were found to support approximately 10^9 to 10^{10} CFU of HPC bacteria per gram of carbon.

Based on the biofilm densities found on GAC fines in the bench scale experiments, and the number of released fines expected in the effluent, application of the homogenization technique would be expected to result in increased recoveries of HPC bacteria if significant numbers of colonized fines were present. However, significant increases in HPC recovery were not seen in post-disinfected pilot plant effluents. From these results it can be concluded that either low amounts of fines were being released from the contactors, the released particles did not support significant attached populations, or the HPC attached to these fines were inactivated during the final disinfection step.

The disinfection kinetics of biologically treated effluents were found to be statistically different than non-biologically treated effluents for both free chlorine and preformed monochloramine. However, the actual level of inactivation was statistically similar for both the biologically and non-

biologically treated streams. Biologically treated streams exhibited lower initial disinfection reaction rates with FAC than the conventionally treated stream. At longer contact times, little additional inactivation was seen for any of the treatment streams. The differences in the initial kinetics of the control stream versus the biological streams, and the tailing off exhibited by all streams was thought to be caused by the presence of a highly chlorine resistant subgroup of the HPC population. Free chlorine resistance at short contact times may be correlated to the degree of biological treatment, as Stream 4, the stream receiving maximum biological treatment, proved to be less amenable to disinfection than the high filtration rate biological stream.

At contact times greater than 30 minutes, preformed monochloramine exhibited a greater tailing-off effect against non-biologically treated than biologically treated effluents. It is hypothesized that the large numbers of HPC bacteria belonging to the highly chlorine resistant subgroup present in the biologically treated effluents were less resistant to combined chlorine than FAC. Because the bulk of the organisms present in biologically treated effluents originate in the filters after preoxidation, sensitivity of these bacteria to monochloramine may also be responsible for the differences in inactivation seen in the biological streams. Unlike the free chlorine results, the two biologically treated streams were not found to be statistically different from each other.

For equal concentrations and contact times, free chlorine was more effective than preformed monochloramine for all treatment streams. However, because of the significant tailing-off effects present for chlorination, the relative efficiency of monochloramine was greater at

longer contact times. For this reason, monochloramine formed by pre-ammoniation or the concurrent addition of chlorine and ammonia, may be a superior final disinfectant for biological drinking water treatment plants.

Further experiments could be conducted to determine if disinfection is more efficient against bacteria attached to GAC fines at the low fines concentrations present in treated effluents, as compared to the high carbon concentrations used in laboratory experiments. Comparison of the homogenized recoveries from samples of sterile water seeded with low numbers of artificially colonized fines, with and without disinfection, could reveal differences in the numbers attached bacteria surviving post-disinfection processes. Low recoveries of attached microbes in the disinfected samples would be an indication that bacteria attached to GAC fines in low concentrations are more susceptible to disinfection than indicated in laboratory studies of GAC media particles present in high concentrations.

Because the disinfection work was conducted at a single disinfectant concentration, investigations conducted at other concentrations and other temperature and pH conditions should be performed to confirm these findings. The speciation of the surviving HPC bacteria from the disinfection experiments conducted during this research would also have been useful. This may have permitted identification of the chlorine resistant subgroups found in the pilot plant effluents. Studies into the effects of pre-disinfection on the selection of native HPC bacteria to colonize the GAC filters would also have been useful in understanding

the prevalence of disinfectant resistant organisms in the biological and non-biological effluents.

TABLES

Table 1: Typical biofilm densities for HPC bacteria

Particle Type	Biofilm Density (CFU/g)	Reference
GAC media*	5×10^7	(Camper et al. 1987)
Anthracite / sand*	5×10^7	(Camper et al. 1987)
GAC media†	10^4	(Camper et al. 1986)
GAC media†	10^8	(LeChevallier et al. 1984)
GAC media*	10^7	(LeChevallier et al. 1988)

* collected from bench scale column

† collected from operating municipal water treatment plant

Table 2: Release of fines from GAC and GAC/sand filters

Released Fines (particles/L)	Size (μm diameter)	Estimated Attached Bacteria in Effluent (CFU/L)	Reference
10 - 62 *	2 - 40	0 - 434	(Stewart et al. 1988)
10 - 30 (<10 μg/L)†	5	10	(DiGiano et al. 1990)

* collected on 10 μm filter from pilot scale GAC contactors pre-oxidized with-ClO_2.

† collected on 0.3μm filter from bench scale GAC/sand filter without Pre-oxidation.

Table 3: Typical distribution system bacteria and their importance

Bacterial Genus	Potential effects
Acinetobacter	Potential rival to other bacterial indicators
Acromonas	Potential pathogen, opportunistic
Alcaligenes	—
Arthrobacter	Coloured water, possible rival
Bacillus	Nitrate reduction, corrosion, rival to other bacterial indicators
Beggiatoa	Rust-coloured water, oxidation of sulfur
Clostridium	—
Corynebacterium	—
Crenothrix	Rust-coloured water (iron bacteria)
Desulfovibrio	Black water, production of H_2S, corrsion
Edwarsiella	Potential pathogen, opportunistic
Enterobacter	—
Escherichia	Indicator of fecal pollution
Flavobacterium	Opportunistic pathogen, rival to other bacterial indicators
Gallionella	Rust-coloured water, corrosion, (iron bacteria)
Klebsiella	Potential pathogen
Legionella	Potential pathogen
Leptothrix	Rust-coloured water (iron bacteria)
Methanomonas	Oxidation of methane
Micrococcus	Nitrate reduction, corrosion, rival to other bacterial indicators
Moraxella	Opportunistic pathogen
Mycobacterium	Potential pathogen
Nitrobacter	Nitrate production, corrosion
Nitrosomas	Nitrate production, corrosion
Nocardia	Potential pathogen
Proteus	Possible rival to other indicators
Providencia	Opportunistic pathogen
Pseudomonas	Opportunistic pathogen, rival to other bacterial indicators

Source: (Maul et al. 1991)

Table 4: Experimental design for the desorption of HPC bacteria from GAC fines released from a bench-scale column

Parameter	-	0	+
Desorption solution*	1 ×	1.5 ×	2 ×
Homogenization speed (rpm)	0	8000	16000
Homogenization duration (min.)	1	3	5
pH	5	7	8

* 0.01 M Tris buffer @ pH 7, 10-6 M Zwittergent 3-12, 10-3 M EGTA, and 0.01% peptone (Camper et al. 1985a).

Table 5: Design matrix for a 2^{4-1} resolution IV factorial

	Coded Level of Parameter			
Trial	**1**	**2**	**3**	**4=123**
1	-1	-1	-1	-1
2	+1	-1	-1	+1
3	-1	+1	-1	+1
4	+1	+1	-1	-1
5	-1	-1	+1	+1
6	+1	-1	+1	-1
7	-1	+1	+1	-1
8	+1	+1	+1	+1

Table 6: Confounding pattern for a 2^{4-1} resolution IV factorial

Effect	Generator: I = 1234 Aliases
β_0	I + 1234
β_1	1 + 234
β_2	2 + 134
β_3	3 + 124
β_4	4 + 123
β_{12}	12 + 34
β_{13}	13 + 24
β_{23}	14 + 23

Table 7: Increases in recovery of HPC bacteria from disinfected and nondisinfected water samples containing suspended colonized GAC fines after the application of a homogenization technique

GAC fines (mg/L)	non-homog. (CFU/mL)	homog. (CFU/mL)	homog. /non-homog.	2 mg/L FAC contact time (min)	colonization time (days)
100	1.4×10^6	2.2×10^6	1.6	0	6
100	1.8×10^4	1.7×10^5	9.1	5	5
100	1.2×10^4	9.9×10^4	8.4	15	5
50	1.2×10^4	5.3×10^4	4.4	15	3
10	1.1×10^4	6.0×10^4	5.4	15	5

Table 8: Increases in recovery of HPC bacteria from non-disinfected PAC sample with PT10 homogenizer probe

PAC Conc. (mg/L)	non-homog. (CFU/mL)	homog. (CFU/mL)	homog./non -homog.
100	3.16×10^5	3.16×10^5	1.00
10	3.16×10^4*	5.50×10^4	1.74
1	3.16×10^3*	6.00×10^3	1.90
.1	3.16×10^2*	1.40×10^3	4.43
.01	3.16×10^1*	1.60×10^3	50.63

* based on 3.16×10^5 CFU/mL multiplied by dilution

Table 9: Increases in recovery of HPC bacteria from disinfected PAC sample with PT10 homogenizer probe

PAC Conc. (mg/L)	non-homog. (CFU/mL)	homog. (CFU/mL)	homog./non -homog.
100	3.70×10^{1}	1.26×10^{3}	34
10	3.70×10^{0}*	5.22×10^{2}	141
1	3.70×10^{-1}*	1.84×10^{1}	49

* based on 3.70×10^{1} CFU/mL multiplied by dilution

Table 10: Increases in recovery of HPC bacteria from non-disinfected PAC sample with PT20 homogenizer probe

PAC Conc. (mg/L)	non-homog. (CFU/mL)	homog. (CFU/mL)	homog./non -homog.
1	4.60×10^3	1.84×10^4	4.01
0.1	$3.45 \times 10^{2*}$	1.63×10^3	4.72
0.01	$2.50 \times 10^{1*}$	2.02×10^2	8.09

* based on actual enumeration by membrane filtration

Table 11: Estimated densities of attached HPC bacteria determined from homogenization of colonized GAC fines.

	Attached HPC (CFU/g carbon)	**Source**
Non-disinfected	8×10^9	Table 7
	1×10^{10}	Table 10
	3×10^8 (GAC granules)	not included
Disinfected	1×10^9	Table 7
	8×10^8	Table 7
	5×10^9	Table 7
	1×10^7	Table 9

Table 12: Increases in recovery of HPC bacteria from disinfected and non-disinfected pilot plant effluent with PT20 homogenizer probe

Treatment Stream	Disinfectant	homog./non-homog.
1	FAC	1.36
1	NH_2Cl	1.68
2	None	1.03
2	FAC	1.34
2	FAC	1.02
2	NH_2Cl	1.36
2	NH_2Cl	1.22
4	FAC	1.13
4	FAC	1.20
4	NH_2Cl	1.18
4	NH_2Cl	1.39

Table 13: Summary of non-linear least squares parameter estimates for simplified kinetic model

Stream		k (min^{-1})	m	SS
FAC:	Filter 1	3.801	0.046	0.66
	Column 2	3.383	0.108	1.49
	Column 4	2.914	0.142	0.83
CAC:	Filter 1	1.062	0.289	1.85
	Column 2	0.526	0.481	2.57
	Column 4	0.550	0.482	0.83

FIGURES

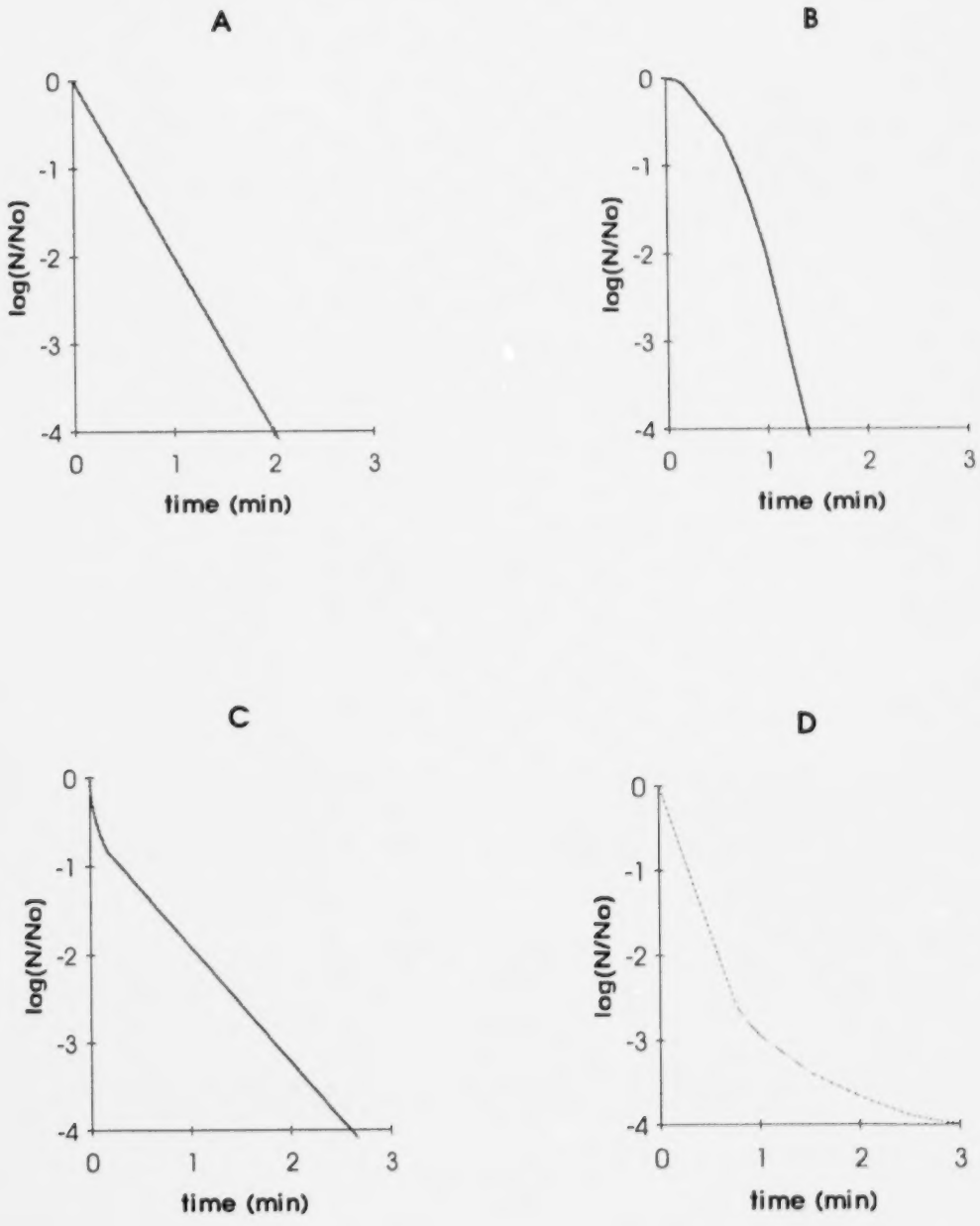

Figure 1: Typical survival curves for disinfection reactions

Source: (Hoff 1986)

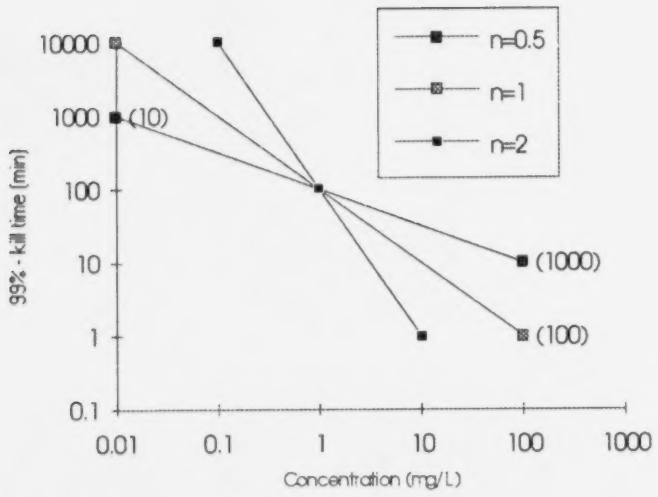

Figure 2: Effect of n value on CT values at different disinfectant concentrations

(CT values given in parenthesis)

Source: (Hoff 1986)

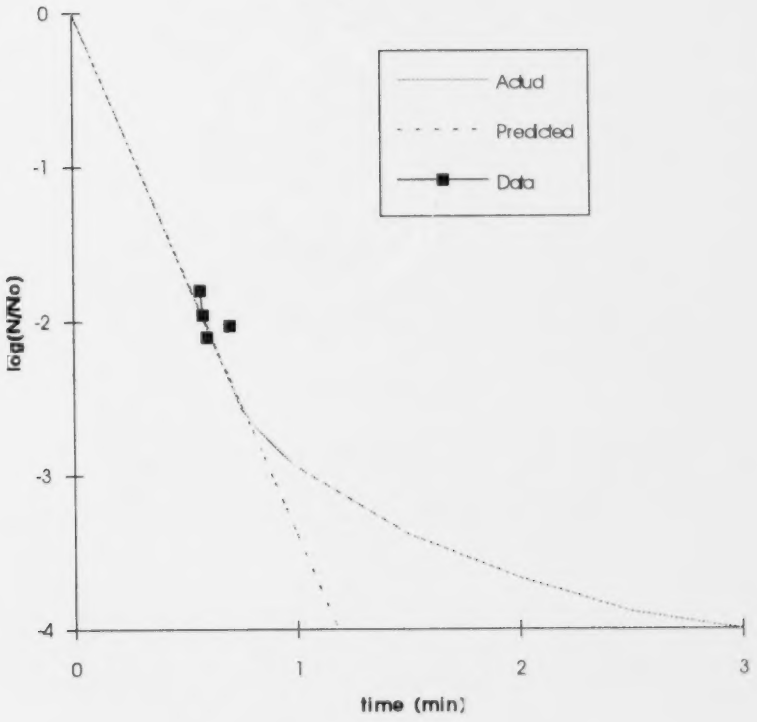

Figure 3: Actual and predicted disinfection kinetics

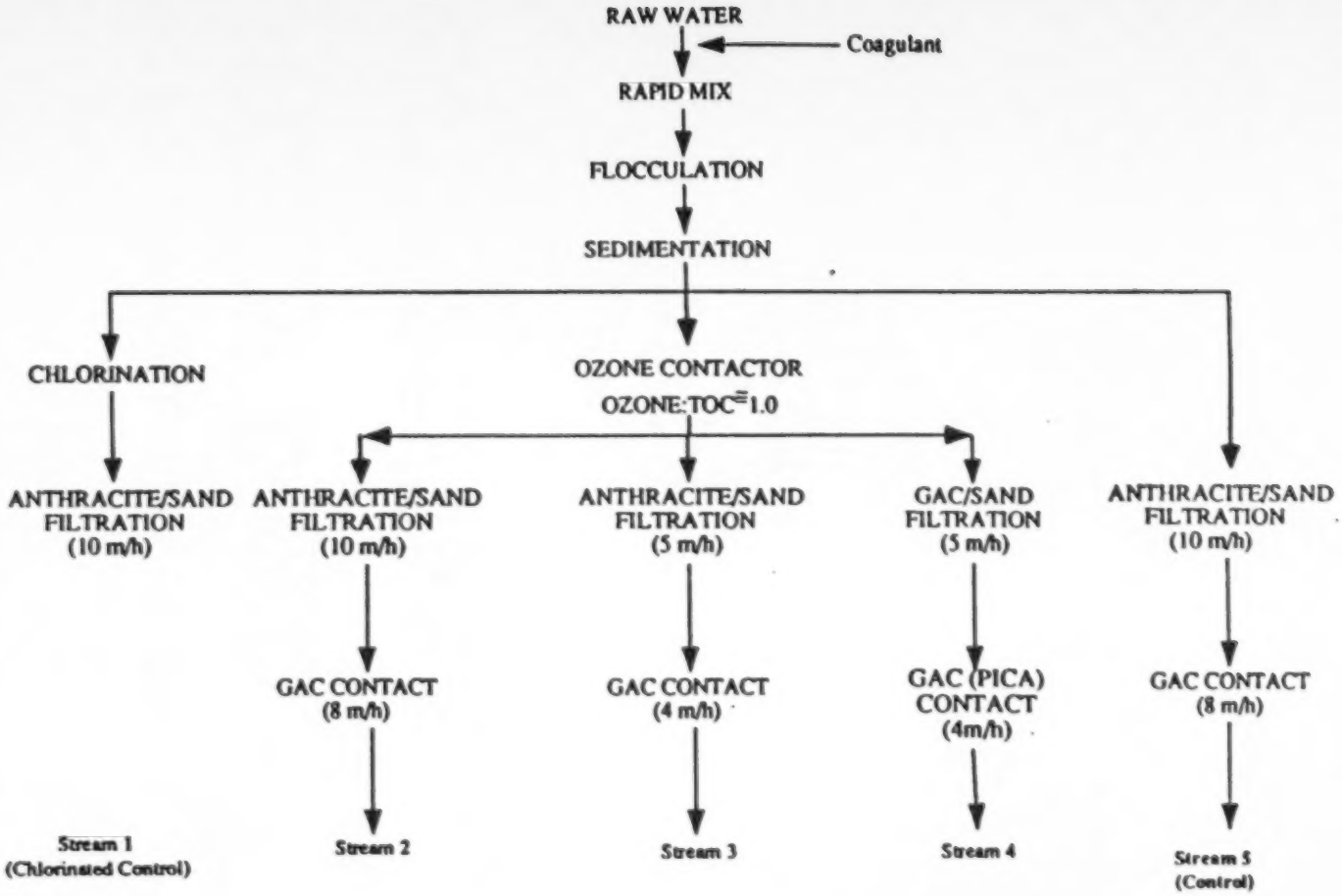

Figure 4: University of Alberta Pilot Plant Configuration for Phase 1

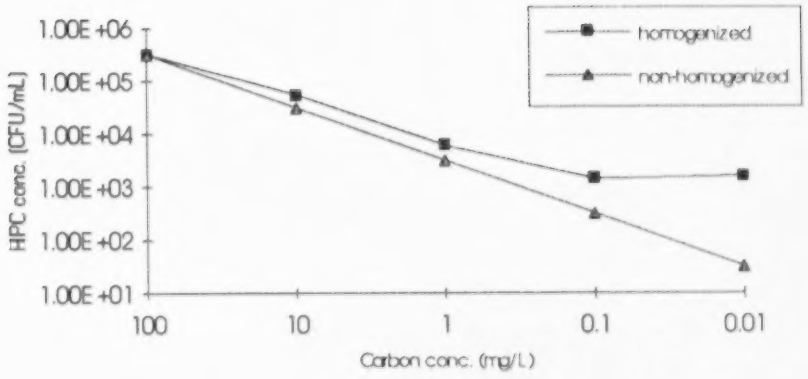

Figure 5: HPC recovery versus carbon fine concentration for non-disinfected PAC sample with PT10 homogenizer probe

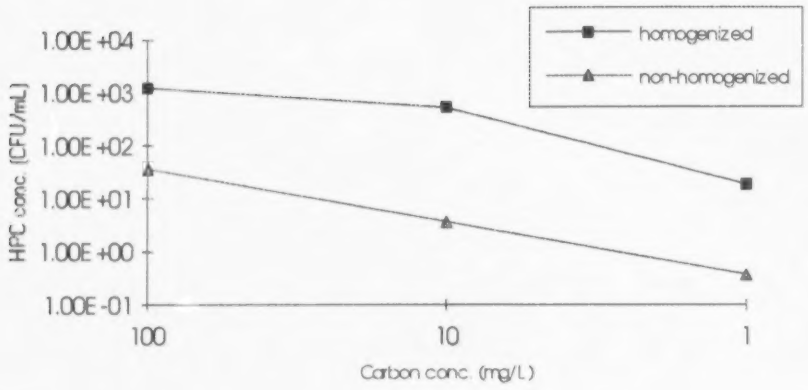

Figure 6: HPC recovery versus carbon fine concentration for disinfected PAC sample with PT10 homogenizer probe

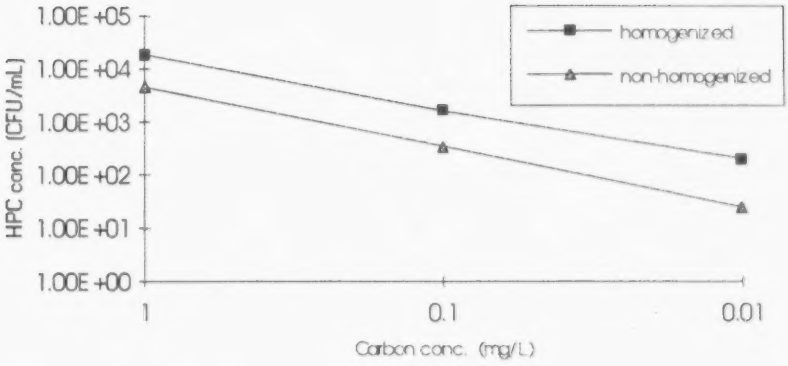

Figure 7: HPC recovery versus carbon fine concentration for non-disinfected PAC sample with PT20 homogenizer probe

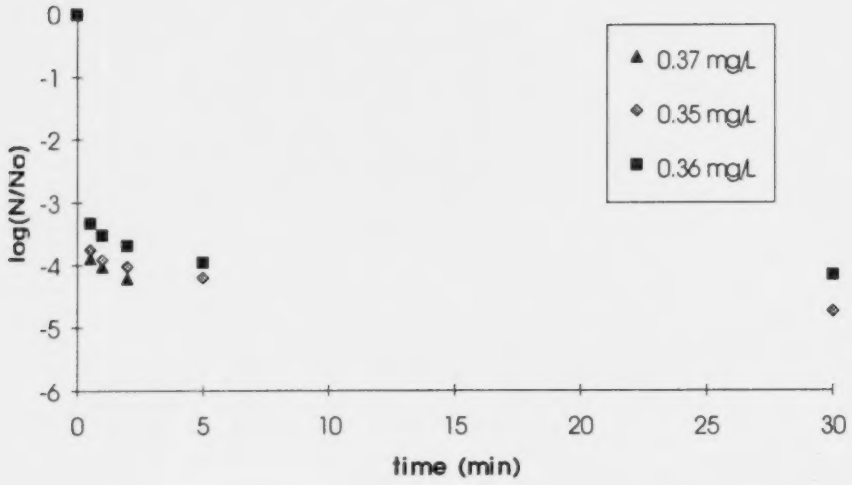

Figure 8: Survival curve for Filter 1 - FAC

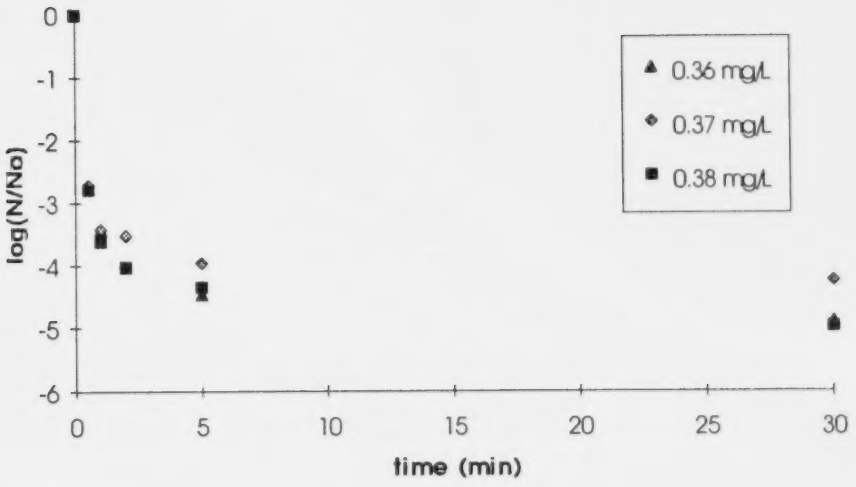

Figure 9: Survival curve for Column 2 - FAC

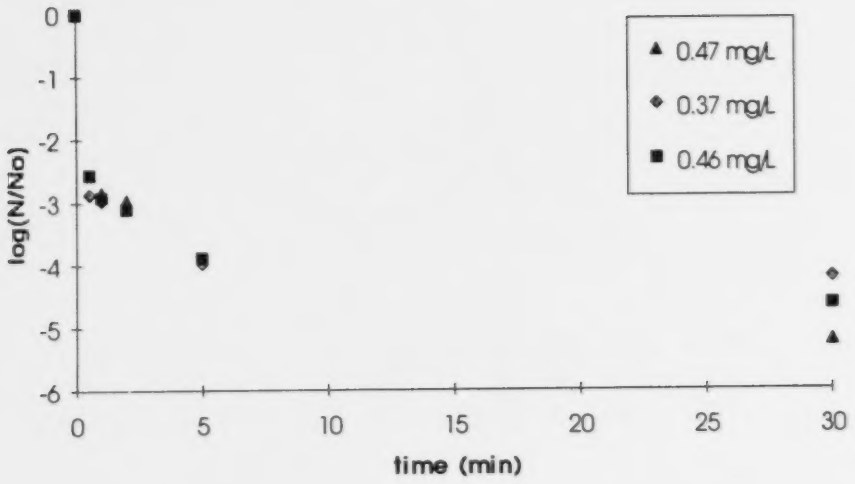

Figure 10: Survival curve for Column 4 - FAC

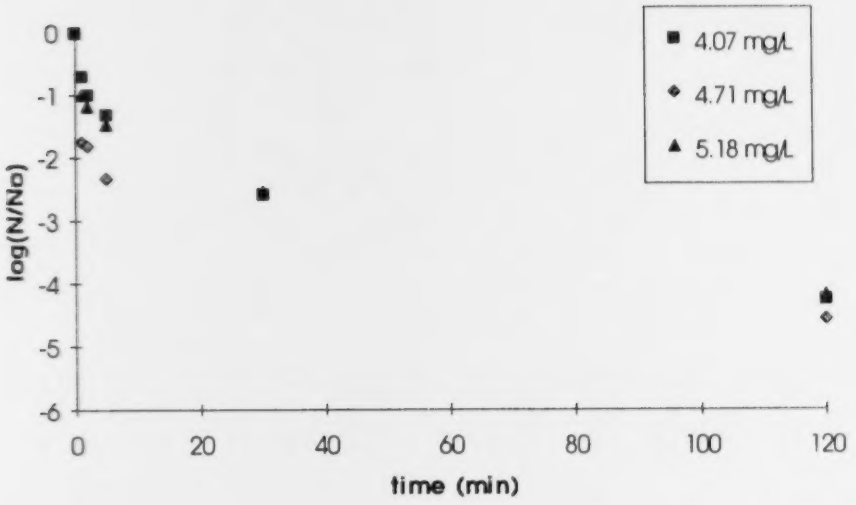

Figure 11: Survival curve for Filter 1 - CAC

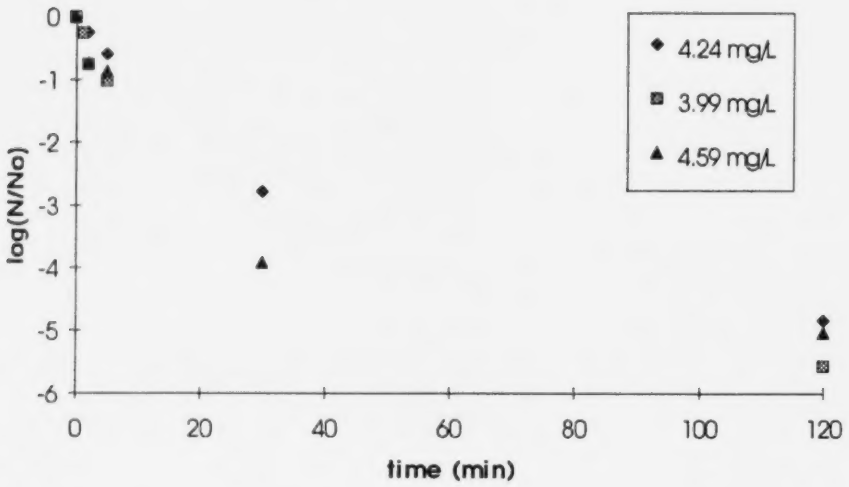

Figure 12: Survival curve for Column 2 - CAC

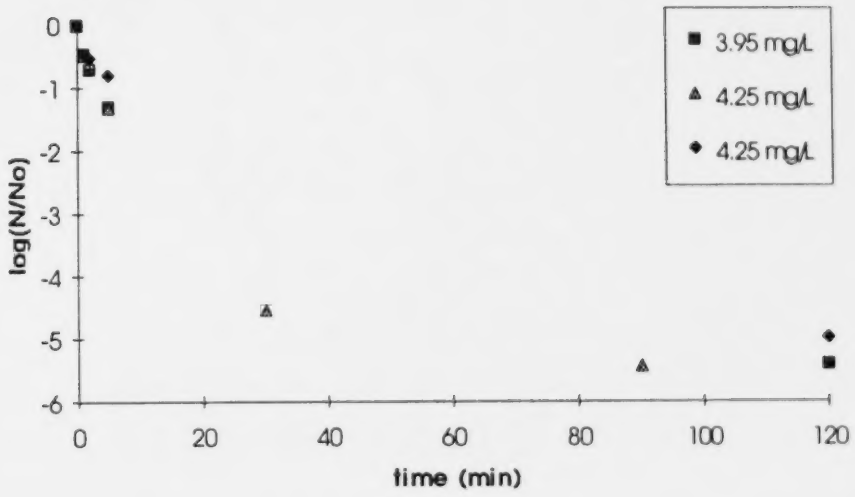

Figure 13: Survival curve for Column 4 - CAC

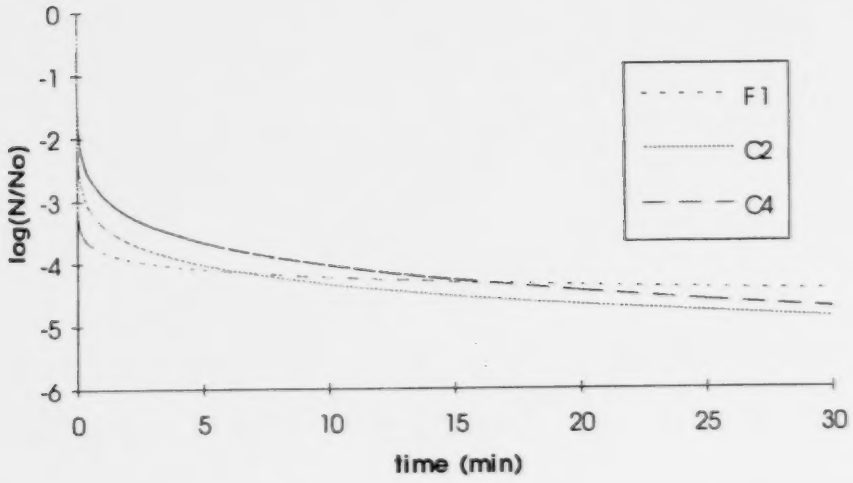

Figure 14: Graphical representation of regression model for FAC

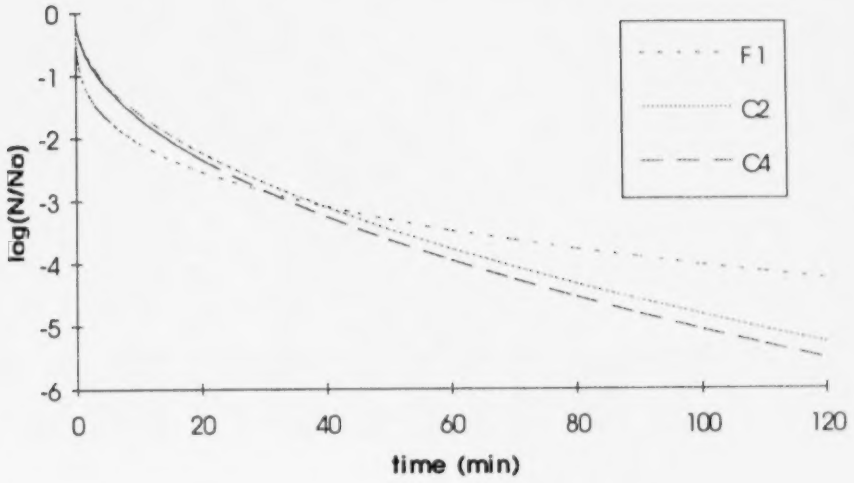

Figure 15: Graphical representation of regression model for CAC

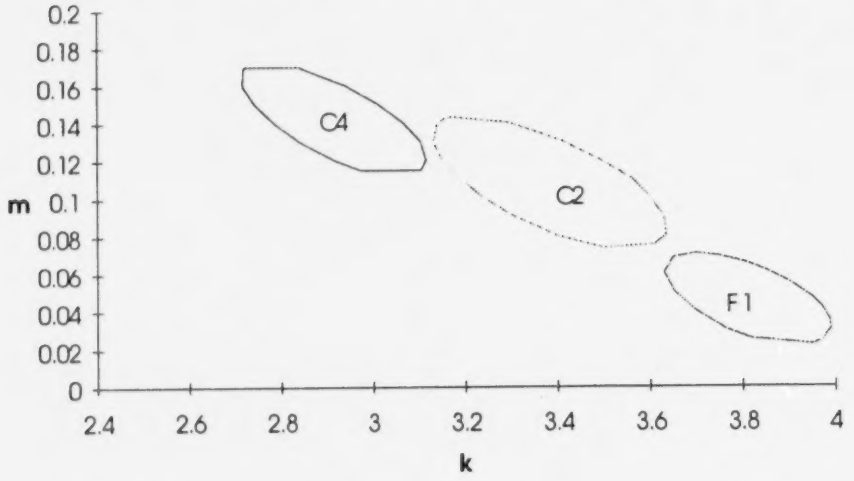

Figure 16: 95% joint confidence region for FAC disinfection

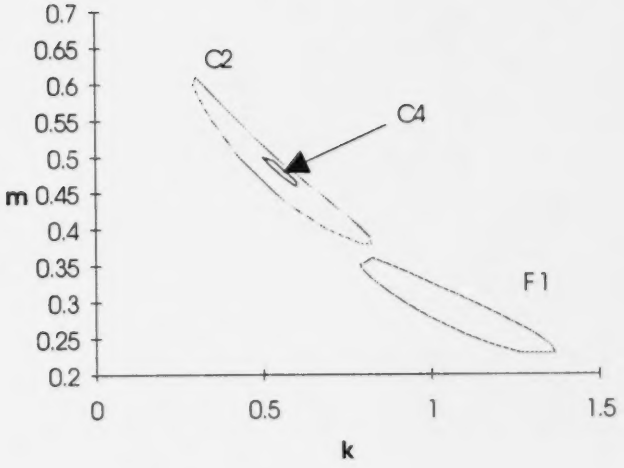

Figure 17: 95% joint confidence region for CAC disinfection

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APPENDIX A: STATISTICAL CALCULATIONS FOR DESORPTION METHODOLOGY EXPERIMENT

AS PERFORMED

Trial No.	const. 0	conc. 1	rpm 2	time 3	pH 4=123	12	13	14	CFU/mL y
7	1	-1	-1	-1	-0.97	1	1	0.97	5.10E+05
5	1	1	-1	-1	1.19	-1	-1	1.19	5.10E+05
2	1	-1	1	-1	1.22	-1	1	-1.22	2.70E+05
3	1	1	1	-1	-0.86	1	-1	-0.86	4.40E+05
4	1	-1	-1	1	1.14	1	-1	-1.14	4.80E+05
1	1	1	-1	1	-1.1	-1	1	-1.1	2.80E+05
6	1	-1	1	1	-0.47	-1	-1	0.47	5.10E+05
9	1	1	1	1	1.25	1	1	1.25	3.80E+05

REPLICATES

Rep	conc.	rpm	min	pH	CFU/mL	d.f.
1	1.5	8000	3	6.67	2.80E+05	
2	1.5	8000	3	6.76	5.00E+05	
3	1.5	8000	3	6.81	4.40E+05	
avg				6.75	4.07E+05	2
SS				0.0101	2.59E+10	
var				0.005	1.29E+10	

HALF NORMAL PLOT

i	|effect|	Pi	Pi%	effect/1000
1	5400	0.07	7.14	5.4004
2	16208	0.21	21.43	16.208
3	18798	0.36	35.71	18.798
4	23400	0.50	50.00	23.4
5	30489	0.64	64.29	30.489
6	54844	0.79	78.57	54.844
7	65361	0.93	92.86	65.361

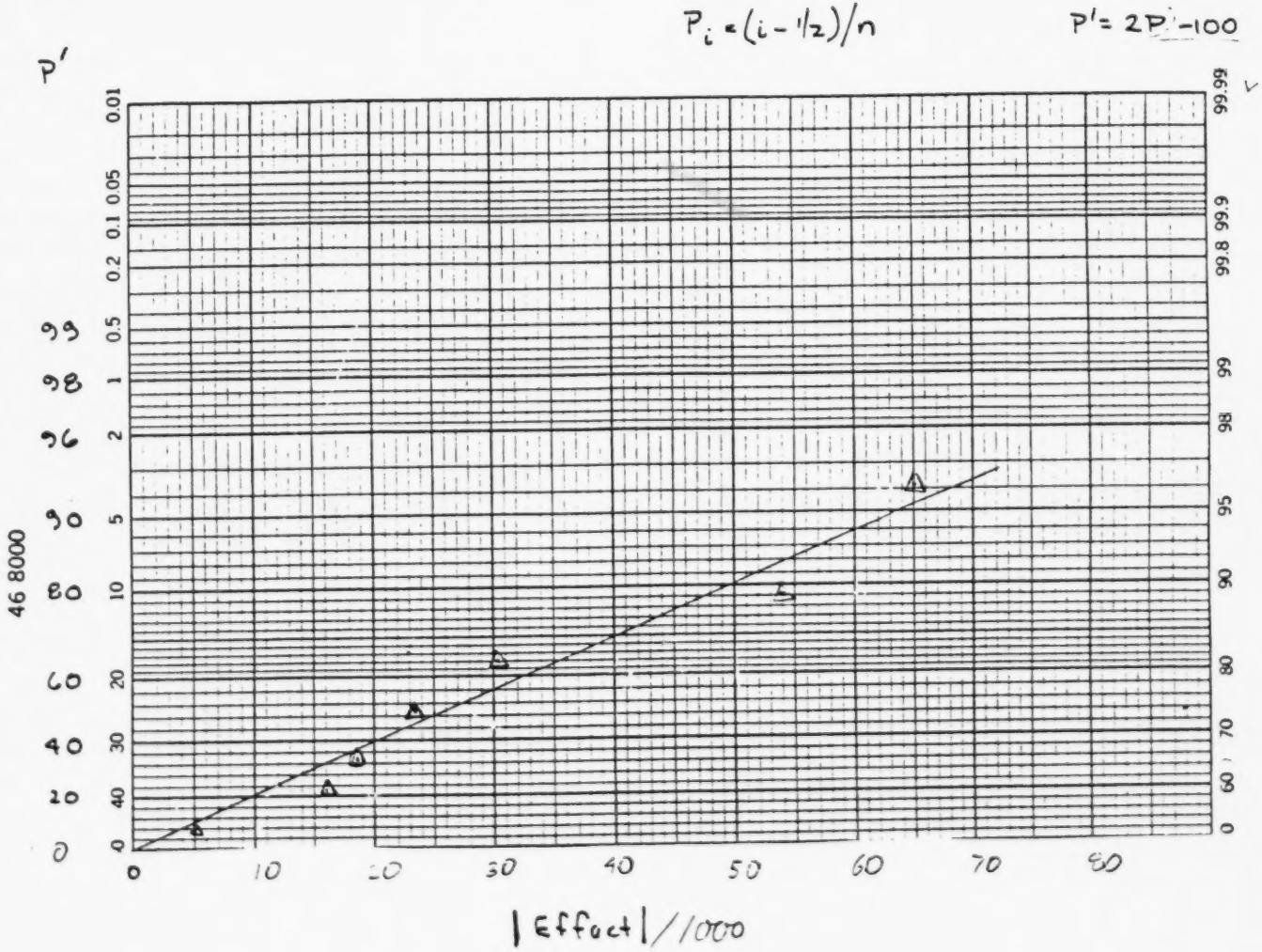
RESULTS

95%, 2 sided, df=8-2=6

t= 2.447

factor	value	95% CI	
constant	428352.87	706637.49	150068.25
concentratio	-30489.23	247795.39	-308773.9
rpm	-18797.55	259487.07	-297082.2
time	-5400.419	272884.2	-283685
pH	-16208.13	262076.49	-294492.8
conc. * rpm	23399.821	301684.44	-254884.8
conc. * time	-65360.94	212923.68	-343645.6
conc. * pH	54844.498	333129.12	-223440.1

none are significantly different than zero


$P_i = (i - 1/2)/n$
$P' = 2P - 100$
P'
0.01
0.05
0.1
0.2
0.5
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
99.8
99.9
99.99
0
10
20
30
40
50
60
70
80
Effect//1000

APPENDIX B: NONLINEAR REGRESSION CALCULATIONS FOR DISINFECTION KINETICS

Summary of Simplified Kinetic Model S=-k*t^m

FAC

Stream	data used	k	n	m	SS	n	F 95%n,p	**SS 95%**
F1	Simplified - All	3.801	N.A.	0.046	0.658	17	3.59	**0.973**
C2	Simplified - All	3.383	N.A.	0.108	1.494	18	3.55	**2.157**
C4	Simplified -no 0.47/5	2.914	N.A.	0.142	0.83	17	3.59	**1.2273**

Data for Parameter Estimates

S=-k*T^m

Filter 1
FAC

k	m
3.810182	0.045817

C	T	obsv. S	Calc S	Residual
0.35	0	0	0	0
0.35	0.5	-3.74473	-3.691081	-0.053646959
0.35	1	-3.91483	-3.810182	-0.104646782
0.35	2	-4.02228	-3.933126	-0.089150649
0.35	5	-4.19837	-4.101758	-0.096609323
0.35	30	-4.75012	-4.452688	-0.29743445
0.366667	0	0	0	0
0.366667	0.5	-3.89086	-3.691081	-0.199774995
0.366667	1	-4.02849	-3.810182	-0.21830693
0.366667	2	-4.21956	-3.933126	-0.286431207
0.366667	30	-4.40723	-4.452688	0.045457992
0.36	0	0	0	0
0.36	0.5	-3.32585	-3.691081	0.365226956
0.36	1	-3.52489	-3.810182	0.285287571
0.36	2	-3.69596	-3.933126	0.237161252
0.36	5	-3.96803	-4.101758	0.133727537
0.36	30	-4.17309	-4.452688	0.279602328
	SS	224.6196	223.96125	**0.65834033**

ANOVA

source	SS	d.f.	MS
model	223.9612	2	111.98062
residual	0.65834	15	0.0438894
total	224.6196		
	n=	17	

Microsoft Excel 4.0 Answer Report
Worksheet: [FACSTAT.XLW]F1FACSS.XLS
Report Created: 5/2/93 9:10

Stream: **Filter 1**
Disinf.: **FAC**
Model: **Simplified**
Data: **all**

Target Cell (Min)

Cell	Name	Original Value	Final Value
E23	SS Residual	65628.3069	0.658340709

Adjustable Cells

Cell	Name	Original Value	Final Value
B3	k	5.040127596	3.809984036
C3	m	1	0.045832847

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
C3	m	0.045832847	C3>=0.001	Not Binding	0.04483285

Data for Parameter Estimates S=-k*T^m **Column 2**
FAC

k	m
3.383429	0.108084

C	T	obsv. S	Calc S	Residual
0.37	0	0	0	0
0.37	0.5	-2.7162	-3.13921	0.423015
0.37	1	-3.43573	-3.38343	-0.0523
0.37	2	-3.52288	-3.64665	0.123767
0.37	5	-3.96302	-4.02629	0.063269
0.37	30	-4.25527	-4.88664	0.631366
0.36	0	0	0	0
0.36	0.5	-2.76264	-3.13921	0.376572
0.36	1	-3.41557	-3.38343	-0.03214
0.36	2	-4.0202	-3.64665	-0.37356
0.36	5	-4.45312	-4.02629	-0.42684
0.36	30	-4.90309	-4.88664	-0.01645
0.38	0	0	0	0
0.38	0.5	-2.79172	-3.13921	0.347487
0.38	1	-3.61334	-3.38343	-0.22991
0.38	2	-4.02286	-3.64665	-0.37622
0.38	5	-4.34803	-4.02629	-0.32174
0.38	30	-4.989	-4.88664	-0.10237
	SS	225.5652	224.0714	**1.4936**

ANOVA

source	SS	d.f.	MS
model	224.0714	2	112.0357
residual	1.493608	16	0.09335
total	225.565		
	n=	18	

Microsoft Excel 4.0 Answer Report
Worksheet: [FACSTAT.XLW]C2FACSS.XLS
Report Created: 5/2/93 8:20

Stream: **Column 2**
Disinfec: **FAC**
Model: **Simplified**
Data: ***All***

Target Cell (Min)

Cell	Name	Original Value	Final Value
$ES24	SS Residual	28613.73581	1.493607738

Adjustable Cells

Cell	Name	Original Value	Final Value
$BS3	k	3.38342859	3.383428595
$CS3	m	1	0.108084378

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
$CS3	m	0.108084378	$CS3>=0.01	Not Binding	0.098084378

Data for Parameter Estimates S=-k*T^m **Column 4**
FAC

k	m
2.914279	0.142459

C	T	obsv. S	Calc S	Residual
0.373333	0	0	0	0
0.373333	0.5	-2.87606	-2.64026	-0.2358
0.373333	1	-2.98435	-2.91428	-0.07007
0.373333	2	-3.09294	-3.21674	0.12379
0.373333	5	-3.97484	-3.66527	-0.30958
0.373333	30	-4.2146	-4.73108	0.51648
0.473333	0	0	0	0
0.473333	0.5	-2.55909	-2.64026	0.08117
0.473333	1	-2.83896	-2.91428	0.07531
0.473333	2	-2.96479	-3.21674	0.25194
0.473333	30	-5.22185	-4.73108	-0.49077
0.456667	0	0	0	0
0.456667	0.5	-2.55072	-2.64026	0.08954
0.456667	1	-2.92224	-2.91428	-0.00796
0.456667	2	-3.11097	-3.21674	0.10576
0.456667	5	-3.88312	-3.66527	-0.21785
0.456667	30	-4.63753	-4.73108	0.09355
	SS	172.2821	171.4519	**0.8305**

ANOVA

source	SS	d.f.	MS
model	171.4519	2	85.72596
residual	0.830488	15	0.055366
total	172.2824		
	n=	17	

Microsoft Excel 4.0 Answer Report
Worksheet: [FACSTAT.XLW]C4FACSS.XLS
Report Created: 5/2/93 8:53

Stream: Column 4
Disinf.: FAC
Model: Simplified
Data: no 0.47/5 min

Target Cell (Min)

Cell	Name	Original Value	Final Value
$ES24	SS Residual	22108.52032	0.830487906

Adjustable Cells

Cell	Name	Original Value	Final Value
$BS3	k	3	2.91427863
$CS3	m	1	0.142458599

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
$CS3	m	0.142458599	$CS3>=0.01	Not Binding	0.132458599

Summary of Simplified Kinetic Model $S=-k*t^m$

CAC

Stream	data used	k	n	m	SS	n	F 95%n,p	**SS 95%**
F1	Simplified -no 4.71/30	1.062	N.A.	0.289	1.85	17	3.59	**2.7355**
C2	Simplified - All	0.526	N.A.	0.481	2.57	16	3.63	**3.9027**
C4	Simplified -no 4 24/30	0.55	N.A.	0.482	1.2	16	3.63	**1.8223**

Data for Parameter Estimates S=-k*T^m **Filter 1**
CAC

k	m
1.061727	0.289849

C	T	obsv. S	Calc S	Residual
4.0725	0	0	0	0
4.0725	1	-0.69699	-1.06173	0.364739
4.0725	2	-0.99474	-1.29797	0.303239
4.0725	5	-1.32275	-1.69281	0.370062
4.0725	30	-2.59263	-2.84548	0.252847
4.0725	120	-4.2606	-4.25267	-0.00793
4.7125	0	0	0	0
4.7125	1	-1.75722	-1.06173	-0.69549
4.7125	2	-1.81858	-1.29797	-0.52061
4.7125	5	-2.33253	-1.69281	-0.63972
4.7125	120	-4.56606	-4.25267	-0.31339
5.18	0	0	0	0
5.18	1	-1.00716	-1.06173	0.054567
5.18	2	-1.17609	-1.29797	0.121883
5.18	5	-1.47549	-1.69281	0.217326
5.18	30	-2.54243	-2.84548	0.303044
5.18	120	-4.18436	-4.25267	0.068304
	SS	89.33155	87.48186	**1.8497**

ANOVA

source	SS	d.f.	MS
model	87.48186	2	43.74093
residual	1.849683	15	0.123312
total	89.33154		
	n=	17	

Microsoft Excel 4.0 Answer Report
Worksheet: [CACSTAT.XLW]F1CACSS.XLS
Report Created: 5/2/93 11:45

Stream: Filter1
Disinf.: CAC
Model: Simplified
Data: no 4.71 / 30 min

Target Cell (Min)

Cell	Name	Original Value	Final Value
E24	SS Residual	2.03139028	1.84968303

Adjustable Cells

Cell	Name	Original Value	Final Value
B3	k	1.139935086	1.061726955
C3	m	0.282490255	0.289849019

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
C3	m	0.289849019	C3>=0.001	Not Binding	0.288849019

Data for Parameter Estimates S=-kT^m

Column 2
CAC

k	m
0.526416	0.481212

C	T	obsv. S	Calc S	Residual
4.2375	0	0	0	0
4.2375	2	-0.25016	-0.73483	0.484669
4.2375	5	-0.5894	-1.14204	0.552644
4.2375	30	-2.78073	-2.70481	-0.07591
4.2375	120	-4.82995	-5.27055	0.440605
3.99	0	0	0	0
3.99	1	-0.2685	-0.52642	0.257915
3.99	2	-0.75696	-0.73483	-0.02213
3.99	5	-1.01223	-1.14204	0.129807
3.99	120	-5.5563	-5.27055	-0.28575
4.59	0	0	0	0
4.59	1	-0.80631	-0.52642	-0.2799
4.59	2	-0.73573	-0.73483	-0.0009
4.59	5	-0.8665	-1.14204	0.275541
4.59	30	-3.91182	-2.70481	-1.20701
4.59	120	-5.03676	-5.27055	0.233789
	SS	106.6266	104.0551	**2.5715**

ANOVA

source	SS	d.f.	MS
model	104.0551	2	52.02756
residual	2.571521	14	0.18368
total	106.6266		
	n=	16	

Microsoft Excel 4.0 Answer Report
Worksheet: [CACSTAT.XLW]C2CACSS.XLS
Report Created: 5/2/93 10:48

Stream: **Column 2**
Disinf.: **CAC**
Model: **Simplified**
Data: **All**

Target Cell (Min)

Cell	Name	Original Value	Final Value
E22	SS Residual	9733.431168	2.571521077

Adjustable Cells

Cell	Name	Original Value	Final Value
B3	k	0.510214113	0.526416433
C3	m	1	0.481211772

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
C3	m	0.481211772	C3>=0.001	Not Binding	0.480211772

Data for Parameter Estimates S=-k*T^m

Column 4
CAC

	k	m
	0.550117	0.482057

C	T	obsv. S	Calc S	Residual
3.95	0	0	0	0
3.95	1	-0.46086	-0.55012	0.08926
3.95	2	-0.71055	-0.76837	0.05782
3.95	5	-1.3091	-1.19508	-0.11402
3.95	120	-5.40369	-5.53018	0.12648
4.24667	0	0	0	0
4.24667	1	-0.4886	-0.55012	0.06152
4.24667	2	-0.59334	-0.76837	0.17503
4.24667	5	-1.3151	-1.19508	-0.12002
4.24667	90	-5.44091	-4.81406	-0.62685
4.25	0	0	0	0
4.25	1	-0.46338	-0.55012	0.08674
4.25	2	-0.53158	-0.76837	0.23678
4.25	5	-0.80371	-1.19508	0.39138
4.25	30	-3.30103	-2.83473	-0.4663
4.25	120	-4.99486	-5.53018	0.53532
	SS	100.5434	99.34027	**1.2028**

ANOVA

source	SS	d.f.	MS
model	99.34027	2	49.67013
residual	1.20284	14	0.085917
total	100.5431		
	n=	16	

Microsoft Excel 4.0 Answer Report
Worksheet: [CACSTAT.XLW]C4CACSS.XLS
Report Created: 5/2/93 11:26

Stream: **Column 4**
Disinf.: **CAC**
Model: **Simplified**
Data: **no 4.24/30 min**

Target Cell (Min)

Cell	Name	Original Value	Final Value
E23	SS Residual	1.517041175	1.202840409

Adjustable Cells

Cell	Name	Original Value	Final Value
B3	k	0.663416354	0.55011741
C3	m	0.448499649	0.482057118

Constraints

Cell	Name	Cell Value	Formula	Status	Slack
C3	m	0.482057118	C3>=0.001	Not Binding	0.481057118

Confidence Interval for Predicted Inactivations

FAC 1 minute contact time alpha=0.05

stream	predicted S	variance	d.f.	t	lower	upper
F1	-3.80998	0.04389	15	2.131	-4.25642	-3.36354
C2	-3.36343	0.09335	16	2.12	-4.03116	-2.7357
C4	-2.91428	0.055367	15	2.131	-3.41571	-2.41285

no streams significantly different

FAC 1 minute contact time alpha=0.10

stream	predicted S	variance	d.f.	t	lower	upper
F1	-3.80998	0.04389	15	1.753	-4.17723	-3.44273
C2	-3.36343	0.09335	16	1.746	-3.91689	-2.84997
C4	-2.91428	0.055367	15	1.753	-3.32676	-2.5018

streams F1 and C4 significantly different

CAC 120 minute contact time alpha=0.05

stream	predicted S	variance	d.f.	t	lower	upper
F1	-4.25267	0.123312	15	2.131	-5.00099	-3.50435
C2	-5.27055	0.183678	14	2.145	-6.18985	-4.35125
C4	-5.53018	0.085914	14	2.145	-6.1589	-4.90146

no streams significantly different

CAC 120 minute contact time alpha=0.10

stream	predicted S	variance	d.f.	t	lower	upper
F1	-4.25267	0.123312	15	1.753	-4.86825	-3.63709
C2	-5.27055	0.183678	14	1.761	-6.02527	-4.51583
C4	-5.53018	0.085914	14	1.761	-6.04635	-5.01401

streams F1 and C4 significantly different